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The Archives of Internal Medicine

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SYPHILIS AND RAYNAUD'S DISEASE *

HANS LISSER, A.B., M.D.
Assistant in Medicine, University of California Medical School,
SAN FRANCISCO

The coincidence of syphilis and Raynaud's disease has occasionally been observed. A few cases have been reported in which the diagnosis of Raynaud's disease was unwarranted, and in which we were dealing with a pure specific obliterative endarteritis; in other cases in which the diagnoses are beyond question, the interdependence is very frail and uncertain, and we might more correctly speak of a simultaneous occurrence; but several instances must be admitted in which the etiologic rôle of an acquired or inherited syphilis is extremely suggestive.

The following case is deemed worthy of record because confusing complications early in the course of the disease obscured the diagnosis, but chiefly because it adds another illustration to this curious group of cases.

History.—The history concerns a colored girl 7 years old, whose present illness began Feb. 5, 1914.

Family History.—The mother has had six miscarriages and a Wassermann test on March 21, 1914, was positive. The father denies lues. A brother and sister of the patient, both younger, seemed normal and showed no luetic stigmata on physical examination.

Past History.—The patient's birth was normal. She had suffered from otorrhea at 2 years of age, and had had spells of earache since then. At 5 years of age she had measles, mumps and whooping cough. Otherwise she had been healthy and normally active.

Present Illness.—Feb. 5, 1914, the mother noticed that the child walked "straddled legged," and examination disclosed a vaginal discharge. Three days later she commenced limping on the right foot and complained of intense pain in her right knee, so that she was confined to her bed, and when seen Feb. 11, 1914, her temperature was 104 F. Vaginal smears showed a Neisser infection. The right knee was swollen, tender, and motion was limited, due to pain. No other joints were involved. Feb. 18, 1914, the patient was admitted to the medical service of the Washington University Hospital. Temperature, 103 F.; leukocytes, 45,000.

Physical Examination.—Forty-six inches long; patient crying, restless, facies show pain, looks ill, is emaciated; muscles small; panniculus absent; complains of cold in a comfortably heated ward. Examination negative except for slightly enlarged tonsils; notched lower incisors; enlarged cervical glands; rapid pulse; and the following interesting condition of the extremities: The right knee

* From the Medical Clinic of Washington University Medical School, St. Louis.

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enlarged and very tender; definite fluctuation; patella floating; left knee not swollen; the right foot shows a black swollen area, including all the foot laterally from the base of the great toe across to the outer side of the heel, including the whole dorsum of the foot as high as the malleoli. There is a sharp line of demarcation with a narrow red margin, especially on the sole. The left foot showed a similar area, extending from between the great and second toes to the middle of the lateral border of the foot, including all four outer toes. Discoloration is dark chocolate over the toes, light brown over the other parts. The ankles are not swollen or tender. The pulse is readily felt in the posterior tibials and dorsalis pedis artery of each foot.

February 20 the Widal test was negative. The right knee was aspirated and about 30 c.c. of a thick purulent fluid removed. Smears showed almost exclusively polymorphonuclear leukocytes. Careful search failed to reveal any organisms and cultures on various media were sterile. Repeated blood cultures were likewise sterile. The fever gradually subsided; the leukocytes falling steadily. Under treatment the vaginal discharge cleared up. The knee was kept in extension from February 24 to March 8 and a roentgenogram of the knee joint taken March 22 showed apparently a normal joint. It seems reasonable to conclude that we were dealing with a gonorrheal vaginitis, complicated by an acute gonorrheal arthritis of the right knee joint.

On admission and for the first four days thereafter, the patient exhibited a peculiar craving for carbohydate foods, constantly crying for bread, crackers and cake, even asking for them when in pain and even at night. The first three days after admission the patient's urine showed a partial reduction of Fehling's solution, an atypical Nylander's test, was fermented by yeast, and polarized light to the right. Repeated examinations since that time have been consistently negative for sugar, even though on one occasion 100 gm. of white bread were given at a single meal. Thus we were dealing with a transient glycosuria probably alimentary.

Two days after admission the Wassermann test gave complete fixation of complement and the patient's serum was again strongly positive April 11 and June 23. With the positive Wassermann of the mother and a history of six miscarriages we are justified in a diagnosis of congenital lues.

We now come to the chief feature of interest, the discoloration of the feet, which eventually developed into a perfectly symmetrical gangrene. Just before admission there had been a spell of very cold weather with considerable snow on the ground. Thinking the condition might be frost bite, careful inquiries were made from the family as to the possibility of exposure to cold. They insisted that the house had been warm and felt reasonably certain that the child had not been in the snow.

Further inquiries elicited the important fact that on Jan. 15, 1914, the patient's left hand became cold and swollen suddenly, the fingers showing a sharply demarcated black discoloration. After two or three days this disappeared only to reappear two days later. This same phenomenon occurred in the hospital, the first time February 24, lasting ten days, with pain, swelling, redness, tenderness and itching of all the fingers going on almost to blackness of the index finger of the left hand. The radial vessels were pulsating; the same condition recurred April 7, clearing up over night.

The process on both feet gradually progressed, the skin becoming ashen gray, finally black, dry, shriveled, the gangrene extending proximally and the line of demarcation assuming a permanent position March 28, almost six weeks after the onset of symptoms. The great toe of the left foot amputated itself April 2, the remainder up to the proximal phalanges dropped off April 15. The process was slower on the right foot, but a symmetrical portion was involved and was removed by a little manipulation June 29. The final result illustrated the wisdom in a case of dry gangrene of permitting a natural demarcation in preference to an amputation. Had a surgical attempt been made it would have

been necessary to sacrifice a great deal more of the feet than the patient was able to save for herself.

Antiluetic treatment was instituted soon after admission and consisted in mercurial inunctions for almost three months. June 29, 0.2 gm. old salvarsan was administered intravenously, 0.25 gm. on July 8, 0.3 gm., July 14, and 0.3 gm., July 29. This therapy had no effect in curing or checking the disease of the feet, and such a result could not be reasonably expected, even if the congenital lues were held responsible for the Raynaud's symptom complex; for the damage was already beyond repair when antiluetic treatment was begun. It is significant to note that an attack of local asphyxia of the fingers occurred April 7, although this attack was much briefer and milder than the previous ones.

DISCUSSION

To summarize, then, the case presents symmetrical gangrene of the feet, and four unquestioned attacks of local asphyxia of the fingers, without exposure to unusual cold. The transient nature of the glycosuria rules out diabetes as the etiologic factor. A gonorrheal vaginitis with probable arthritis occurred, and a gonorrheal septicemia with emboli might be held responsible for the gangrene of the lower extremities; but the question of such sepsis was never proved and does not seem probable clinically. The patient had congenital syphilis, and an obliterative endarteritis could cause the condition. But the vasomotor attacks of the fingers, combined with the symmetrical gangrene of the feet is so typical of Raynaud's disease that the latter diagnosis must be very seriously considered. In fact it seems justifiable to conclude that the patient simultaneously suffered from three diseases—gonorrheal vaginitis with arthritis, congenital syphilis and Raynaud's disease. It remains to discuss what relation, if any, existed between these maladies. Were we dealing with a pure time coincidence, a simultaneous occurrence without interdependence, or did the syphilis, or the gonorrhea, or both, act in relation to the Raynaud's symptoms as the *"Auslösendes Moment?"*

It is not within the scope of this paper to include more than a brief summary of the literature. A complete bibliography of those publications which specifically consider the relation between syphilis and Raynaud's disease has been added for those more fully interested. Raynaud's disease is uncommon, yet its occurrence is not so extremely infrequent as to relegate it to the category of medical rarities. Cassirer saw 56 cases, and including a careful review of the literature up to 1911, has gathered together over 300 unquestioned instances of the disease. Coming now to the group of cases in which an hereditary or acquired lues is present, we find a considerable discrepancy in the figures published. Monro would allow only 2.8 per cent., while Castellino and Cardi find 22 cases of syphilis in 306 cases of Raynaud's disease. Probably the proportion is still higher. The appended 54 references contain more than 22 cases. Perhaps 10 per cent. will not be too high an estimate.

Raynaud himself seemed not to attach any importance to the presence of syphilis, though he mentions an observation to that effect by Portal (1836) and Henry (1857). Defranc notes an early record of this combination described by Liston in 1836, before Raynaud had written his treatise. As Cassirer correctly points out, Morgan in 1889 was probably the first to direct attention to the possible interdependence of syphilis and Raynaud's disease. Cases illustrating this coincidence in acquired lues, have been reported by Amann, Klotz, Morgan, Castellino and Cardi, Elsenberg, Fordyce, Giovanni, Germer, Jacoby, Morton, Nash, Ornellas, Puzey, Riva, Tounton, Balzer and Fouquet, Lustgarten, Cassirer, Phelps, Schuster, Gaucher, Gougerot and Meaux Saint Mare, and Semon. To these must be added those cases of congenital lues, exhibiting a Raynaud's symptom complex, namely, the reports of Hutchinson, Marsh, Humphrey, Krisowski, Young, Wherry, Dyce Duckworth, Pasteur, Rietschel, Schiff, Spieler, Stoltzner, Glaser, Brocq (quoted by Gaucher), Bosanyi, Durantes, Beck, and the case that forms the basis of this report.

As regards the pathologic anatomy of Raynaud's disease, Dehio's statement in 1893 obtains today, namely, that none of the published pathologic investigations, where negative findings have been reported, are complete and detailed enough to justify the conclusion that this malady has no recognizable lesions. The positive findings include changes in the nervous system and circulatory system, either separately or in both. But such changes have been very inconstant, and their etiologic relationship to the Raynaud's disease rather indefinite, while the majority of cases autopsied have failed to reveal any lesions whatsoever. The theories as to the pathogenesis of the disease are briefly:

THEORIES AS TO PATHOGENESIS

1. The theory of Raynaud himself. He would classify the syndrome as a central vasomotor neurosis, due to direct or reflex stimuli on the vasomotor center. The majority of writers agree in this conception, modifying it only in certain details.

2. There are those (Pitres and Noesske, etc.) who insist that a peripheral neuritis is the cause of the disease.

3. In disease of the peripheral arteries some have found their etiologic agent.

4. Concerning a case of acquired lues with Raynaud's disease, Gaucher, Gougerot and Meaux Saint Mare offer the following explanation: Either vascular spasm, caused by a syphilitic peripheral neuritis or a central vasomotor disturbance, as the primary feature, a secondary endarteritis from prolonged vasoconstriction sufficing to complete the clinical picture; or more probably, a primary luetic endarteritis, remain-

Appearance of gangrenous foot in author's case.

Illustrating Article by Hans Lisser, A.B., M.D.
The Archives of Internal Medicine, October, 1915.

ing latent symptomatically until a spasm set up through the nerve channels determines the onset of local asphyxia and subsequent gangrene.

5. Some authors (Hochenegg, Fuchs) find it necessary to assume a special as yet unknown trophic center, through disease of which or the nerves leading from which, Raynaud's symptom complex follows.

6. Bearing syringomyelia in mind, some are tempted to seek a similar origin, in the spinal cord for Raynaud's disease (Bender, Tscherback, Lyle and Grieve). Oppenheim would suggest that a group of cases at least are due to lesions of the posterior and lateral columns.

7. Still others (Moebius, Solis-Cohen, Levi, Rothschild) ascribe a causal rôle to the glands of internal secretion, especially the thyroid.

It is worth noting that Raynaud's symptom complex has been observed as a complication in some fifty different diseases, including acute and chronic infectious diseases, intoxications, mental and nervous maladies, disorders of the circulatory system and ductless glands, etc. These widely divergent types of disease cannot all be responsible for the unique and singular syndrome of Raynaud. This very fact is one of the most suggestive arguments in support of its being a clinical entity. That Raynaud's disease (probably 10 per cent. of all cases) occurs very frequently in syphilitic patients, might superficially suggest an intimate relationship. However, this statistical preponderance is not surprising, in view of the prevalence of syphilis. A similar case might be made out for tuberculosis. This frequency, then, can have no weight in establishing any relationship between the two diseases.

In discussing the coincidence of these diseases, we can suppose the following possibilities:

1. The symmetrical gangrene and, more important, the other signs and symptoms, may be caused directly by a syphilitic lesion of blood vessels or the nerve supply to such blood vessels. For the final proof of such a contention, the *Spirochaeta pallida* must be demonstrated in the lesion, just as the gonococcus has been found in the tissues of a gonorrheal arthritis, or the spirochete by Noguchi in the cords of tabetics and brains of paretics. Probably many cases of myocardial weakness were suspected of a luetic origin, even though no gummatous process was histologically discernible; but positive statement of fact had to await Warthin's demonstration of the spirochete in the heart muscle.

2. Raynaud's symptom complex may be caused indirectly by the syphilitic toxin. The number of conditions in which this toxin is the etiologic agent are becoming increasingly appreciated. We are coming to recognize a larger number of arthropathies as luetic, even though

the histology is not characteristically syphilitic, nor the signs and symptoms sufficiently typical to permit invariably of a correct diagnosis. Clinicians are becoming convinced that lues of the lung is not as rare as hitherto supposed. We have come to regard an aortitis confined to the arch of the aorta as almost always luetic, even though no gummatous change is present at necropsy. In the conditions mentioned, the history of syphilitic infection, the Wassermann reaction and the response to specific therapy have been the basis for the diagnosis. To what extent do these standards apply to the combination, lues and Raynaud's disease? Some cases of Raynaud's disease have been ascribed to a luetic basis, merely because of a positive Wassermann or history. This is quite unjustifiable. The positive Wassermann and history merely indicated that the patient had syphilis, but not that the Raynaud's disease was an expression of active syphilis.

Those who defend an interdependence between the two diseases advance as their strongest argument, the disappearance of the Raynaud's signs and symptoms under vigorous specific medication. Some of the most striking instances are those reported by Krisowski, Morgan and Bosanyi. Naturally such cases are extremely suggestive, but are they conclusive? It would seem preposterous to doubt such evidence, and yet when one recalls how suddenly and without warning a local asphyxia or local syncope may disappear, a certain skepticism may be permitted as to the rôle played by the specific therapy. In those cases in which local syncope and asphyxia never occurred, and merely a symmetrical gangrene was favorably influenced by mercury or salvarsan, we may actually question the diagnosis of Raynaud's disease and wonder whether a specific endarteritis was not the pathologic basis of the gangrene.

3. We may suppose that syphilis may so lower the bodily resistance as indirectly to make the individual more susceptible to Raynaud's disease. If this were the only relation between the two diseases it would be the same and no more important than the influence of the many other acute and chronic infectious diseases, intoxications, etc., in which Raynaud's syndrome has been observed as a complication.

Morgan concludes concerning his case: "that Raynaud's symptoms appeared as manifestations of tertiary syphilis." Bosanyi concludes similarly concerning his two cases: "Raynaud's disease can develop as a symptom of hereditary lues." But the final proof of such a point of view is still lacking.

It is a pleasure to acknowledge the kindness of Professor Dock in permitting me to report this case.

516 Sutter Street.

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A STUDY OF ATRIOVENTRICULAR RHYTHM FOLLOWING AURICULAR FLUTTER*

PAUL DUDLEY WHITE, M.D.
BOSTON

A cardiac rhythm arising in the atrioventricular junctional tissues in the human is a rare and usually very transient affection, so uncommon in fact that it has never been the object of careful investigation. Premature beats and paroxysms of tachycardia having their origin in the junctional tissues have been reported frequently. Belski[1] in 1909 wrote of six cases of acute infectious disease in which polygrams seemed to indicate that the bradycardia was not infrequently due to an atrioventricular rhythm. In one case the subcutaneous injection of atropin sulphate, gram 0.001, produced in twenty minutes an acceleration of the pulse from 42 to 137, while the rhythm apparently changed from one arising in the atrioventricular junctional tissues to one of the normal sino-auricular type. Five hours after the atropin had been given the pulse had dropped to 55 and the rhythm was still normal. The next day at a rate of 46 the polygram again showed atrioventricular rhythm.

Williams and James[2] reported in 1914 a patient showing a reversal of the cardiac mechanism in whom atropin produced no change. In October, 1913, Hume,[3] writing on the polygraphic study of four cases of diphtheria, discussed two cases which seemed to show atrioventricular rhythm for a few days just preceding auricular flutter and death. In one of these the pulse rate during the *a-v* rhythm was high (98 to 100), in the other 68. In the latter case the sino-auricular node examined postmortem was inflamed and the muscle fibers were in a state of granular degeneration while the node of Tawara and the bundle of His were almost normal.

Experimentally atrioventricular rhythm in the animal's heart has been produced and studied by several investigators, notably by Thomas Lewis.[4] He has shown that when the sino-auricular node is destroyed

* Submitted for publication May 24, 1915.
* From the Wards and Outpatient Department of the Massachusetts General Hospital.
* Reported at the Peter Bent Brigham Hospital, Boston, March 9, 1915.

1. Belski, A.: Ztschr. f. klin. Med., lxvii, 1909.
2. Williams, H. B., and James, H.: Heart, 1914, v.
3. Hume, W. E.: Heart, 1913, v.
4. Lewis, Th.: Heart, 1913-1914, v

or sufficiently depressed by cold or vagal stimulation, the atrioventricular node assumes the rôle of pace-maker for the heart. Its automatic rate is lower than that of the sino-auricular node, but higher than that of the lower levels of the atrioventricular bundle as found at times in complete heart block. A rapid rhythm arising in the atrioventricular junctional tissues as in some cases of paroxysmal tachycardia is probably due to irritation and not to the normal rate of stimulus production in this area of the heart.

Experimentally one may find in atrioventricular rhythm that the intervals between auricular systole and ventricular systole vary from a positive value shortened below that of normal rhythm to a negative value, depending partly on the site of the stimulus production in the junctional tissue and partly on the resistance to conduction between (a) node and ventricle and (b) node and auricle. If the pacemaker lies low in the node there may presumably be a definite interval between ventricular systole and auricular systole—i. e. a negative *as-vs* value (Fig. 21). Or if the auriculonodal junction is abnormally resistant to the passage of impulses, the negative *as-vs* interval may presumably occur even with the pacemaker in the central part of the atrioventricular node. Lewis, White and Meakins[5] have shown experimentally that in the cat the auriculonodal junction is the most susceptible point of the *a-v* conducting system when asphyxiation is used as the test-method.

Since by the electrocardiogram *P* represents auricular systole and *R* the onset of ventricular systole, we may have in atrioventricular rhythm values varying from shortened *P-R* intervals to definite *R-P* intervals. In a similar way venous tracings would show conduction intervals varying from shortened *a-c* intervals to those of a *c-a* sequence.

The present case which I report is exceptional for the following reasons:

First, the patient was suffering from an auricular .flutter with a ventricular rate of 130, with no discomfort other than slight dyspnea on exertion and with no realization that there was anything at all wrong with his heart.

Secondly, instead of returning to a sino-auricular rhythm following the fibrillation of the auricles, which is the frequent story of the course of the "flutter heart" under treatment, this patient underwent a transition from fibrillation to a rhythm arising in the atrioventricular junctional tissues.

5. Lewis, Th., White, P. D., and Meakins, J.: Heart, 1914, v.

Thirdly, this atrioventricular rhythm has established itself so well that it presents an excellent opportunity for study.[6]

Details follow:

History.—W. S., a single man of 37 years, a teacher, entered the nerve service of the Massachusetts General Hospital under the charge of Dr. E. W. Taylor on Jan. 8, 1915. He came for the relief of "weakness in his legs" and "obesity." His admission diagnosis was "tabes" (?).

Family and Past History.—Father, aged 71, confined in a hospital for the insane. Mother, one brother and two sisters living and well. Two sisters dead — diphtheria and pneumonia. Patient has had pertussis and rarely a sore throat. Never rheumatism or chorea. Severe attack of typhoid fever with relapse nineteen years ago. Right ankle sprained at age of 9 years. Patient was always stocky and stout and played football in youth. After typhoid fever nineteen years previously his legs became much smaller and his weight "settled in his abdomen."

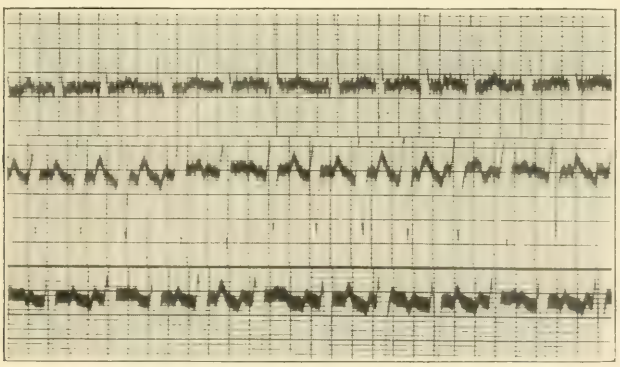


Fig. 1.—Electrocardiogram showing the three leads of Einthoven in auricular flutter with rapid ventricular action. Taken Jan. 10, 1915. In this as in the remaining electrocardiograms the ordinate represents 10^{-4} volt. Abscissa represents time interval of 0.2 second. Curves in this and following illustrations reduced to two-thirds size of originals.

History of Present Complaint.—About nine years previously patient noticed gradual onset of weakness in his right knee and ankle. He would often get tired on walking but in 1907 he climbed a height of 2,800 feet in Maine without discomfort. For the past three years he has fallen down about once a month, due to his "knee giving out." No dizziness, nausea, vomiting or headache. He has been unable to carry anything heavy for years — cannot now

6. April 12, 1915, the rhythm was continuing unchanged, ten weeks after its onset. He was examined frequently in this period. May 11, 1915, the patient again showed auricular flutter by electrocardiogram, with an auricular rate of 300 and an irregular ventricular response at a rate of 100 per minute. The patient observed a sudden change in his pulse from a slow, regular rate to an irregular and much more rapid rate on the 10th of May, the day before the electrocardiogram above noted was taken, and fourteen weeks after the onset of the atrioventricular rhythm. May 18 an electrocardiogram showed again the atrioventricular rhythm restored through the administration of 1.8 grams of digitalis leaves in the course of a week. A bigeminy similar to that previously described was present.

carry a suitcase. He has great trouble going up and down stairs — his knees fail to support him and he has to pull himself up by the hand rail. No loss of sphincteric control. For several years some dyspnea on exertion, ascribed by patient to his obesity. No cough or expectoration. No edema.

Habits: Tobacco: four to five cigarettes daily; an occasional pipe. No alcohol. Venereal diseases denied.

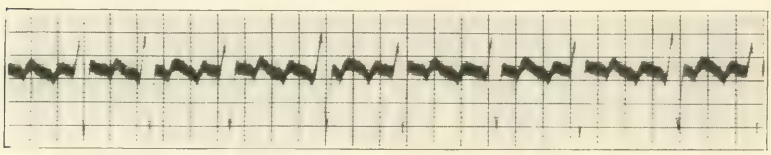


Fig. 2.—Electrocardiogram. Lead II. Auricular flutter.

Physical Examination.—Hair scanty, but a moderate amount occurs on the body (axillae, chest, abdomen, pubes). Mucous membranes of good color. Pupils slightly irregular but react normally. Tongue protruded straight and shows no tremor. No lead line on gums. Tonsils not enlarged.

Lungs showed slight dulness at left apex front and back with prolonged expiration. No râles. Spine straight, not tender.

Heart: Accurate percussion impossible because of thick chest wall but heart seems somewhat enlarged. Apex not felt. No murmurs. Sounds of fair quality. Rhythm at times irregular.

Abdomen obese; otherwise normal. Liver not enlarged to percussion or palpation. Genitalia normal.

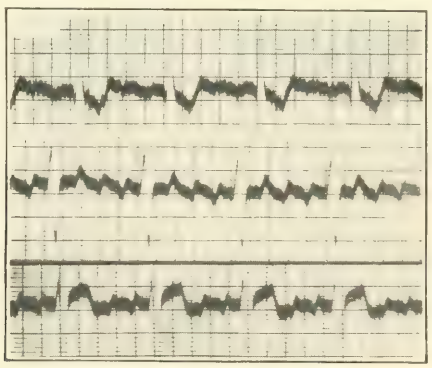


Fig. 3.—Leads I, II and III. Auricular flutter with 4:1 *a-v* block. Taken Jan. 18, 1915.

Extremities show muscular weakness.

Reflexes: Right knee-jerk not obtained; left weak. No ankle-clonus, Babinski or Oppenheim. Romberg's sign absent. Abdominal and cremasteric reflexes normal. Arm reflexes not obtained.

Gait: "Slaps feet down and is hesitant, not ataxic."

Clinical Observations.—Temperature as a rule subnormal (never above 99 F. while in the ward). Respiration 20 to 24. Pulse rate at wrist dropped steadily from 120 on entrance to 38 on discharge (nineteen days later).

Blood pressure: 125 mm. Hg, systolic; 92 mm. Hg, diastolic.

Urine: Ounces 20 to 55 daily. Two examinations showed a clear acid urine of 1.022 gravity without albumin, sugar, bile or acetone bodies; rare hyaline cast in the twenty-four-hour sediment.

Blood: White cells = 6,300 per c.mm. Red cells = 4,240,000 per c.mm. Hgb. = 85 per cent. Smear normal. Wassermann test negative.

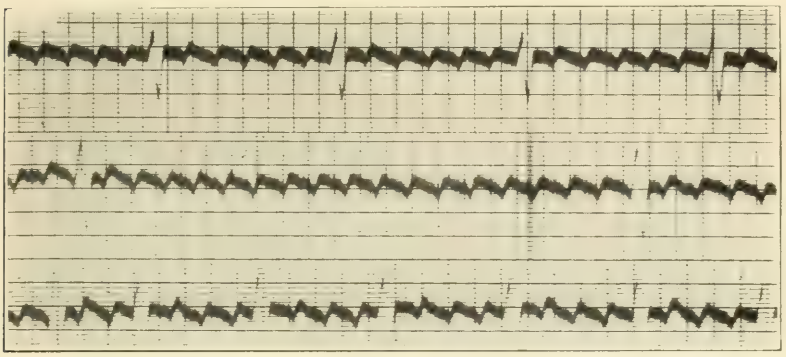


Fig. 4.—Lead II with right vagal pressure. Upper and middle curves represent effect of vagal pressure, 6:1 and 18:1 block, respectively. Lower curve shows the electrocardiogram following the vagal pressure.

Roentgen-Ray Examination.—January 11. Skull, "medium sized sella turcica with apparent bridging of clinoids." January 22. (Chest at 7 feet from tube.) "Apex of heart is in the sixth interspace, 11.5 cm. to left of median line. Right border is 6.4 cm. to right of median line. Total transverse diameter is 17.9 cm. Greatest transverse diameter of great vessels 8 cm. Length of heart 18 cm."

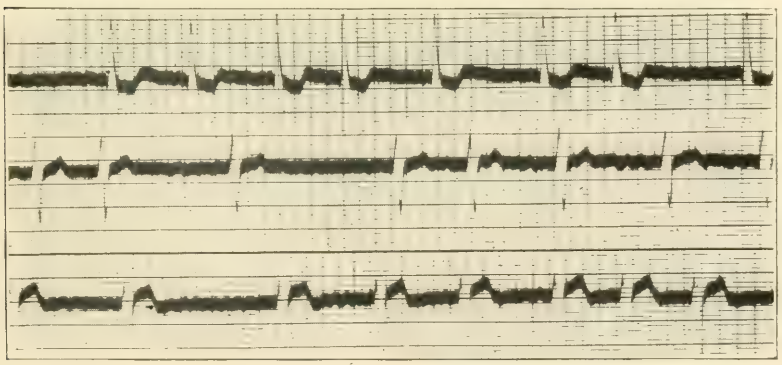


Fig. 5.—Leads I, II and III. Auricular fibrillation. Taken Jan. 19, 1915.

Weight fell from 183 to 177¼ pounds on a fat-free diet. Fields of vision normal. Two attempts to have the patient take levulose for a tolerance test were unsuccessful on account of vomiting.

Drugs.—Aside from one dose of morphin solution on January 8, no drugs were given other than pills of Caesar and Loretz digitalis leaves, gram 0.1, t. i. d. for eight days, the patient receiving 2.4 grams from January 12 to 19, inclusive. Patient was discharged Jan. 27, 1915, nineteen days after entrance.

Electrocardiograms.—The Cambridge model of the Einthoven string galvanometer with rotary time-marker was used.

January 9, the day after the patient's admission to the hospital, the irregularity of the pulse was brought to my attention. A radial pulse tracing taken then showed that flutter of the auricles was probably present. The next day, January 10, the first electrocardiogram was taken and the series of plates showed that auricular flutter was in progress (Figs. 1 and 2). Also there is indica-

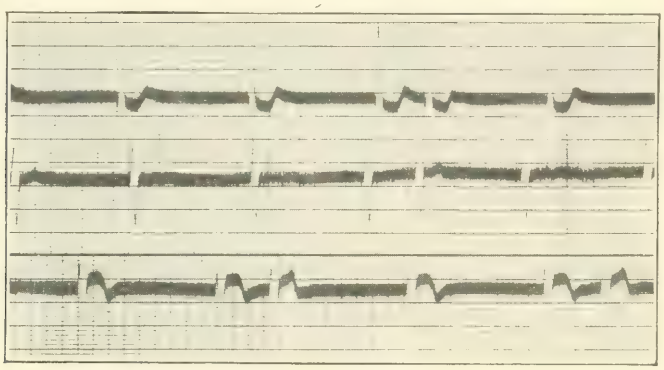


Fig. 6.—Leads I, II and III. No evidence of auricular activity. Taken in a. m., Jan. 23, 1915. Ventricular rate = 43 per minute.

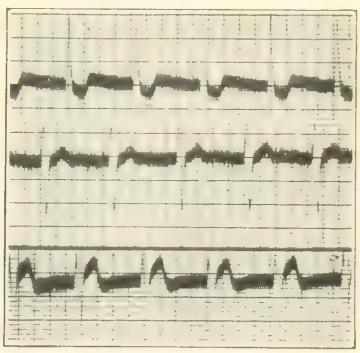


Fig. 7.—Leads I, II and III. No evidence of auricular action. Taken in p. m., Jan. 23, 1915, same day as that on which the record shown in Fig. 6 was obtained. Rate much higher, 70 per minute.

tion from Leads I and III and from the width of the ventricular complex that conduction in the right branch of the *a-v* bundle is defective. The auricular rate varied from 285 to 292 on January 10; on the 15th it was 250 to 252, on the 16th 272 and on the 18th 244, a range of 47 contractions per minute. The ventricular rate dropped from 130 on the 10th to 96 on the 11th, 67 on the 15th, and 61 on the 18th. At this latter rate there was a clear 4:1 *a-v* block (Fig. 3). At the higher rates the degrees of block were irregular. Vagal pressure (especially on the right side) increased the block definitely, on the

15th of January producing a pause in the pulse of over four seconds and equivalent to an 18:1 block (Fig. 4).

On January 19 auricular fibrillation was discovered (Fig. 5). The ventricular rate was 50 and irregular. The electrocardiographic oscillations representing the fibrillation of the auricles occurred at about the rate of 450 per minute. The next day the condition persisted but the oscillations were smaller. The

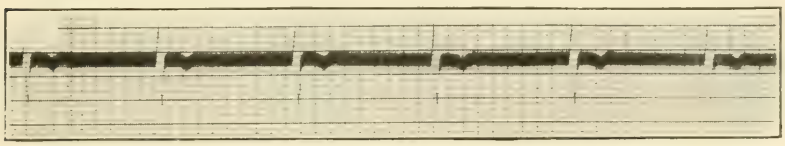


Fig. 8.—Lead II. No evidence of auricular action. Taken Jan. 25, 1915.

ventricular rate then was 38 to 40. On January 23, three days later, the ventricular rate was 43, in general much more regular, at times bigeminal, while the diastolic portion of the electrocardiogram was almost smooth (Fig. 6). On the afternoon of the same day the electrocardiogram showed a rate of 70 (Fig. 7), the ventricular complexes almost equidistant, varying at times only a few thousandths of a second; there was no sign of auricular systole. The condition at this time may have been one of idioventricular rhythm without auricular contraction (either because of a very fine auricular fibrillation or of a total standstill of the auricle) or there may have been at this time an atrioventricular rhythm with the auricular complex concealed somewhere in the ventricular. The fine oscillations in the first and second leads are due to the patient's tremor, which in the third lead has almost disappeared.

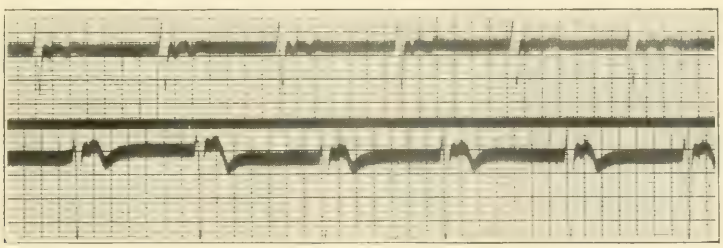


Fig. 9.—Leads II and III. First evidence of invert auricular complex following the initial ventricular complex. Taken Jan. 30, 1915.

January 25, two days later, the same situation occurred, there being direct evidence of a regular ventricle systole only (Fig. 8). Inversion of the I deflections appeared here for the first time. The intervals between ventricular complexes on the electrocardiogram vary less than two-hundredths of a second. Diastole is smooth.

On January 30 a new feature appeared in the electrocardiogram, an inverted deflection immediately after the *S* and preceding the *T* (Fig. 9). This deflection is interpreted as the index of auricular systole. It appears most distinctly in Leads II and III. The reasons for considering that this is the auricular complex are as follows: (a) the deflection appears in an inverted form at a brief interval after the regularly occurring *R* wave and is represented by a definite wave in the jugular pulse (Figs. 19 and 20); (b) it is subject to certain conditions to be mentioned below, and (c) there is no other evidence in systole or diastole of auricular action. Williams and James[2] by means of the fluoroscope

were able to see the contraction of the auricle in their case of "Reversal of the Cardiac Mechanism," although it was impossible to time its contraction in relation to the ventricular beat. In the present instance, with the help of Dr. George W. Holmes, a similar examination was made and the auricle and ventricle seemed to contract synchronously.

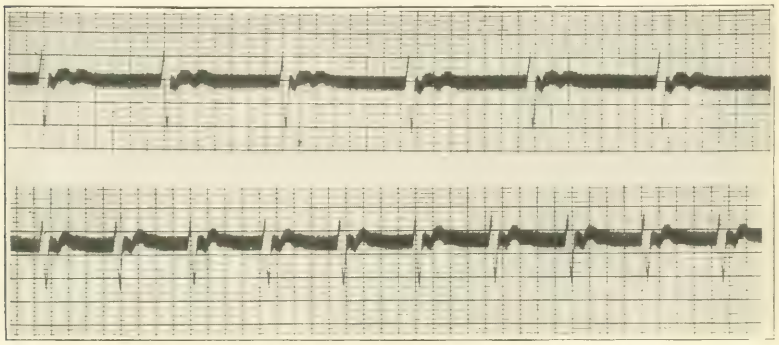


Fig. 10. Lead II. Showing the effect of atropin, gram 0.002 injected subcutaneously. Upper record taken before the injection, lower record taken sixty minutes after the injection. Rate increased from 42 to 66. Note the change of the *T* wave after atropin. Taken Feb. 10, 1915.

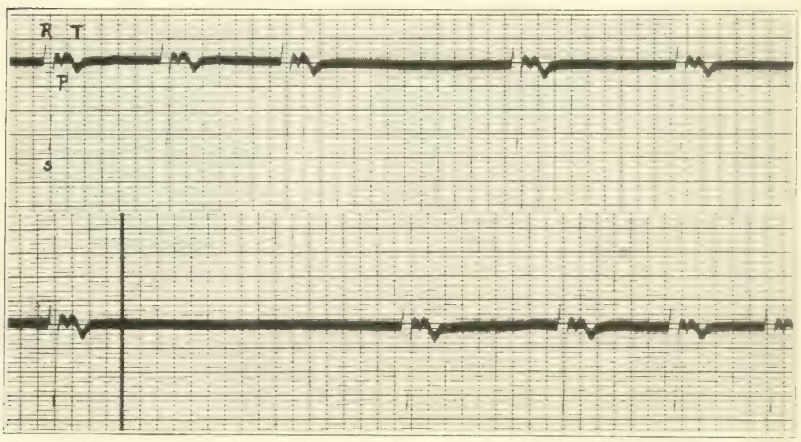


Fig. 11.—Lead III. Showing the effect of right vagal pressure. In upper curve pressure was begun shortly after second beat, in lower curve pressure was begun when the plate began to travel.

The fact that the ventricular complex has not changed from its character while responding to auricle except with regard to the *T* deflection, makes it evident that there persists here a supraventricular rhythm which is not induced by auricular systole. If the excitatory process does not arise in the ventricular musculature or in the auricles, it must have its origin in the atrioventricular junctional tissues perhaps as low as the left branch of the *a-v* bundle. The reversal of the interval *as-vs* to *vs-as* would indicate that the excitatory process arising

somewhere in the *a-v* junctional tissues and traveling down to the ventricles and up to the auricles reaches the former first either because of the distal position of the pacemaker in the *a-v* tissue or because of the resistance in the auriculonodal junction, or from both causes (Fig. 21). To test this a further course of digitalis, 2 gm. of the leaves in one week, was given while the patient was showing the atrioventricular rhythm. Except for a slight lengthening of the *R-P* interval there was no change after 1 gm.; but on the completion of 2 gm. the electrocardiogram showed no longer any definite auricular deflection. A condition of backward block from the *a-v* node affords a likely explanation of this phenomenon, the *P* being absent or buried in the *T* (Fig. 13). Experimentally the auriculonodal junction has been shown to be the susceptible joint in *a-v* conduction as mentioned above.

In this case the considerable variation in the length of the *R-P* interval (from 0.123 to 0.420 sec.) under different conditions, with regularly recurring ventricular contractions, is evidence here against a mechanical origin of the auricular complex resulting from the ventricular contraction, such as has been suggested by Cohn and Fraser[7] in their case of block in which frequently inverted auricular complexes followed directly on the ventricular.

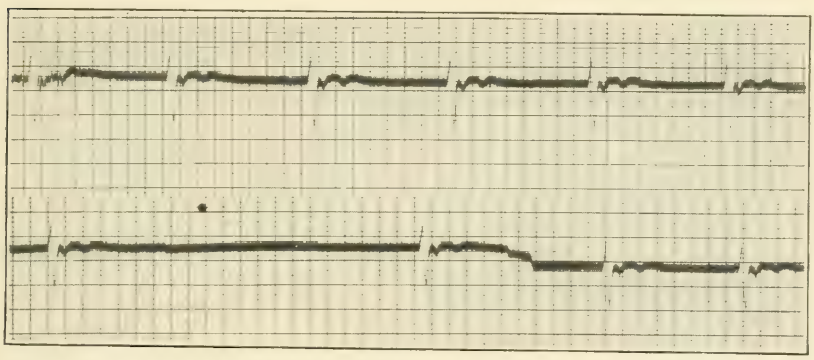


Fig. 12.—Lead II. Showing the effect of left vagal pressure. Upper record taken before the vagal pressure, lower record during the vagal pressure. Taken Feb. 15, 1915.

INFLUENCE OF VARIOUS FACTORS ON THE ATRIOVENTRICULAR RHYTHM

Rate.—Daily variation. The table of pulse rates shows well the daily variation of the pulse, which was slow in the morning just before breakfast, and usually more rapid after the evening meal. This variation accords in general with that seen in sino-auricular rhythm. The one occasion (Feb. 27, 1915) on which the morning rate was 72 was apparently due to the fact that the preceding night had been badly interrupted by vomiting and abdominal distress. After spending the day in bed the pulse dropped to 44. The average morning and evening pulse rates for fifty-five days were 40 and 45, respectively. It should be noted that the patient lives quite comfortably with his atrioventricular rhythm and is up and about every day.

7. Cohn, A. E., and Fraser, F. R.: Heart, 1914, v.

Exercise. Exercise increased the rate. Repeated lifting of the arms produced a quickening amounting to but a few beats while the exertion of climbing one flight of stairs increased the rate from 32 to 57 in one instance, and from 46 to 54 on another occasion, the pulse keeping perfectly regular. After resting one half hour following further exercise (walking about) on the first occasion the rate became 42.

Table of Pulse Rates

1915	a. m.	p. m.	1915	a. m.	p. m.
1/25	38	46	3/13	40	48
1/26	38	44	3/14	42	42
1/27	38	..	3/15	36	44
1/30	..	43	3/16	38	40
2/ 6	..	47	3/17	50	52
2/10	..	42	3/18	40	38
2/11	34	52	3/19	42	34
2/12	32	50	3/20	44	36
2/13	32	50	3/21	36	40
2/14	37	46	3/22	38	36
2/15 *	32	58	3/23	38	42
2/16	32	45	3/24	32	50
2/17	..	58	3/25	36	48
2/18	42	46	3/26	36	54
2/19	46	50	3/27	50	46
2/20	46	48	3/28	42	42
2/21	36	50	3/29	38	42
2/22 †	32	44	3/30	..	50
2/23	58	50	3/31	36	50
2/24	38	56	4/ 1	44	48
2/25	54	46	4/ 2	..	44
2/27	72	44	4/ 3	46	54
2/28	44	46	4/ 4	34	40
3/ 1	38	45	4/ 5	36	42
3/ 5	40	48	4/ 6	48	40
3/ 6	46	58	4/ 7	38	48
3/ 7	46	40	4/ 8	36	44
3/ 8	34	36	4/ 9	36	34
3/ 9	32	46	4/10	36	40
3/10	34	40	4/11	40	40
3/11	48	44	4/12	40	..
3/12	36	50			

* Digitalis started. † Digitalis stopped.

Respiration. Quiet respiration had very little effect on the rate of the atrioventricular rhythm, but forced respiration produced definite results, corresponding to the respiratory arrhythmia of the normally beating heart. These observations were made with the Mackenzie ink polygraph. The rates during four periods of forced expiration were 42, 43, 43, and 41 beats per minute, while during the periods of forced inspiration preceding the periods of forced expiration, the rates were 48, 52, 55 and 50, respectively—an average of 42 beats per minute during forced expiration and of 51 beats per minute during forced inspiration.

Atropin sulphate injected subcutaneously in the dose of 0.002 gram did not restore sino-auricular rhythm. It was thought that the sino-auricular node might have been depressed by vagal influences (as had probably happened in Belski's case) but such was not the situation. The atrioventricular rate, however, responded to the withdrawal of the vagal influences (Fig. 10), and in fifteen minutes rose from a regular rate of 42 to one of 68. The high point was thirty minutes after the injection when the electrocardiogram showed a rate of 71. Thereafter the rate gradually dropped, and next morning registered 34.

Vagal pressure produced marked decrease in the rate of the heart during its atrioventricular rhythm, moderate right sided pressure slowing the heart from a rate of 50, so that at one time a pause of over

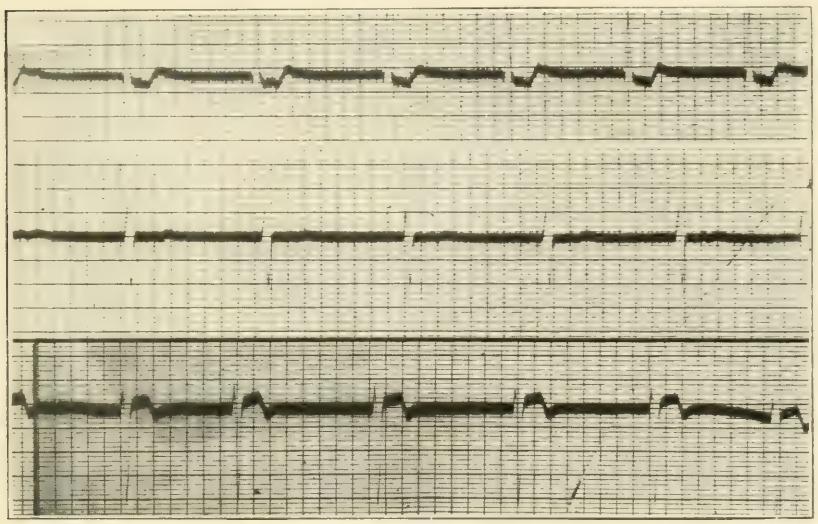


Fig. 13.—Leads I, II and III. Showing the effect of 2 grams of digitalis leaves in one week. Taken Feb. 23, 1915.

four seconds occurred (Fig. 11). The left vagus acted in the same way on moderate pressure, the heart which was beating at 37 stopping for over four seconds (Fig. 12). Ocular pressure was tried on both sides but the results were less striking than in the case of vagal pressure. The ocular pressure was not exerted strongly enough to cause severe pain. Experimentally Lewis[8] found that the right vagus appears to have a larger control upon *a-v* rhythm than does the left, but in some animals he found the reverse true. In the present case, so far as can be judged with regard to rate, both vagi acted similarly. Lewis' conclusion that the influence of both vagi is usually more pro-

8. Lewis, Th.: Heart, 1914, v.

found, relatively and absolutely on the *a-v* rhythm than on the *s-a* rhythm, finds indirect confirmation in the human subject here, for although there has been no sino-auricular rhythm to test in this patient, I have not seen any person with normal rhythm not under the influence of digitalis who responded so readily to slight vagal pressure as did this individual. The fact that the sino-auricular node did not escape during the marked slowing of the atrioventricular rhythm in this patient by vagal pressure would suggest that this node had ceased to function.

Digitalis: This drug, in a dosage of 2 gm. of the leaves during the period of one week, had practically no effect on the rate of the atrio-

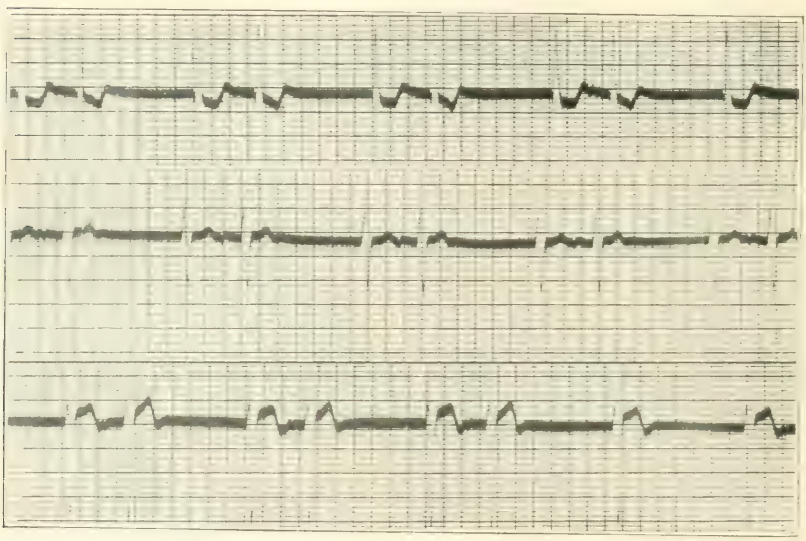


Fig. 14.—Leads I, II and III. Showing the bigeminy present on Feb. 24, 1915, two days after stopping digitalis. Before injection of atropin.

ventricular rhythm (Fig. 13), although a bigeminy occasionally appeared after it had been taken. The day after the last of the digitalis was administered the ventricles were beating regularly at a rate of 38 without any clear evidence of auricular complex in the electrocardiogram (Fig. 13). The next day the auricular deflection reappeared and a bigeminal pulse occurred (Fig. 14). Atropin (0.002 gm.) was injected subcutaneously, an acceleration of the pulse to 80 followed three hours later (Fig. 16). It was thought that in this way the auriculonodal block might be released. The *R-P* interval became definitely shorter and the bigeminy constant at first (Fig. 15), but later when the rate was fixed at 80 the auricular complexes disap-

peared. An electrocardiogram taken at this time showed a remarkably uniform ventricular rate, the range in eight successive beats being but 0.007 second (from 0.748 to 0.755 second). Beside the retardation and disappearance of the auricular complex in the electrocardiogram, other evidence of toxicity from digitalis were headache and anorexia.

Variation of the R-P Interval.—In order to determine this interval with some degree of accuracy the electrocardiographic plate was projected on a glass screen at a magnification of twenty-five times. Measurements of the various intervals were then made by means of calipers and a scale with divisions of 1/60 inch. Comparison of the interval measurements was made with the measurements of the fifths

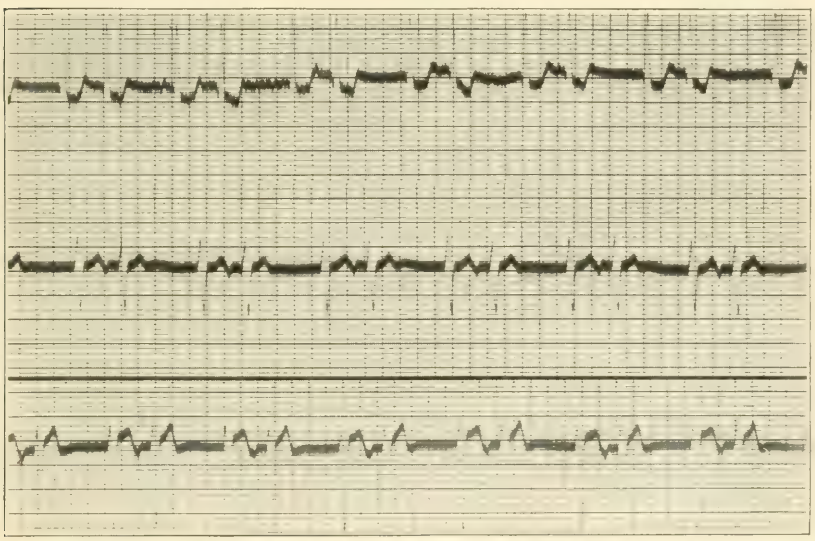


Fig. 15.—Leads I, II and III. Bigeminy still present one and one-half hours after subcutaneous injection of atropin, gram 0.002, Feb. 24, 1915. Note increase in rate and decrease in *R-P* and *P-R* intervals.

of a second marked off by the vertical lines on the plate. In this way determinations could be made to thousandths of a second, the maximal range of one of the measurements itself done by three different individuals being 0.002 second. Taking all causes of inaccuracy into consideration I find that the maximal error amounts to less than one-hundredth of a second. The onset of the *P* deflection was often not sharp, but the notch itself was always clearly marked and quite uniform. Therefore, having determined the onset-to-notch interval in the most definite plate, a correction was made on this basis from the notches found in the other plates measured.

The "normal" range of the *R-P* interval on different days was very considerable, from 0.123 second to 0.235 second, but from beat to beat the interval varies at the most but a few thousandths of a second when not influenced by some unusual factor.

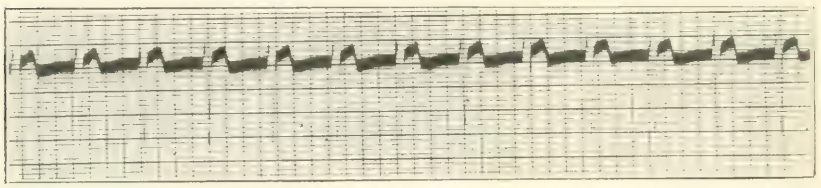


Fig. 16.—Lead III. Taken three hours after atropin, Feb. 24, 1915. Auricular complex no longer evident.

Vagal pressure: Pressure over the right vagus increased the *R-P* interval on one occasion from 0.143 second to 0.173 second and on another occasion from 0.231 to 0.253 second, the continuation of the pressure increasing the prolongation to 0.332 second coincident with

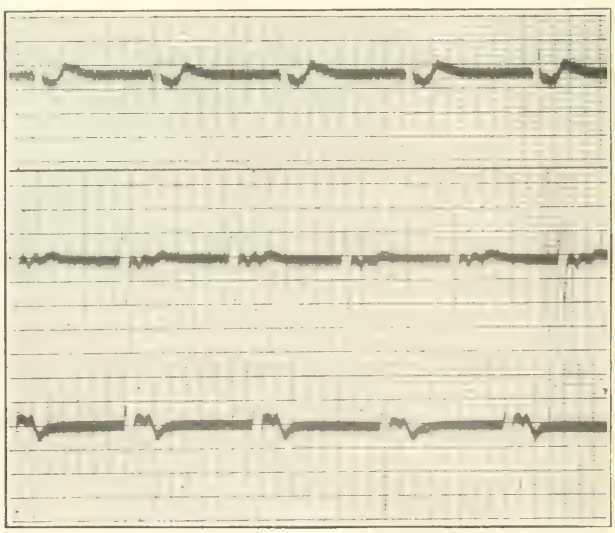


Fig. 17.—Leads I, II and III. Taken March 1, 1915, one week after cessation of course of digitalis.

a marked slowing of the whole heart. On one occasion during left vagal pressure the *R-P* interval increased from 0.149 to 0.156 second.

Digitalis: After 1 gm. of digitalis leaves the *R-P* interval measured 0.155 second; after 2 gm. the *P* disappeared (Fig. 13). Two days after stopping the digitalis the interval measured 0.378 to 0.420 second

(Fig. 14); one week after the drug was omitted the interval was 0.217 second (Fig. 17).

Atropin: The sulphate injected subcutaneously in the dose of 0.002 gm. when the *R-P* interval was 0.131 to 0.134 second caused a prolongation of the interval to 0.162 and 0.166 second (Fig. 10). The explanation of this retardation is not clear. It is probably due to the increasing difficulty of backward conduction with increase in rate (here the *a-v* rate rose from 42 to 71). Two days after stopping the digitalis atropin was again given, while the *R-P* interval was long (0.378 to 0.420); the interval was at first much shortened—nearly

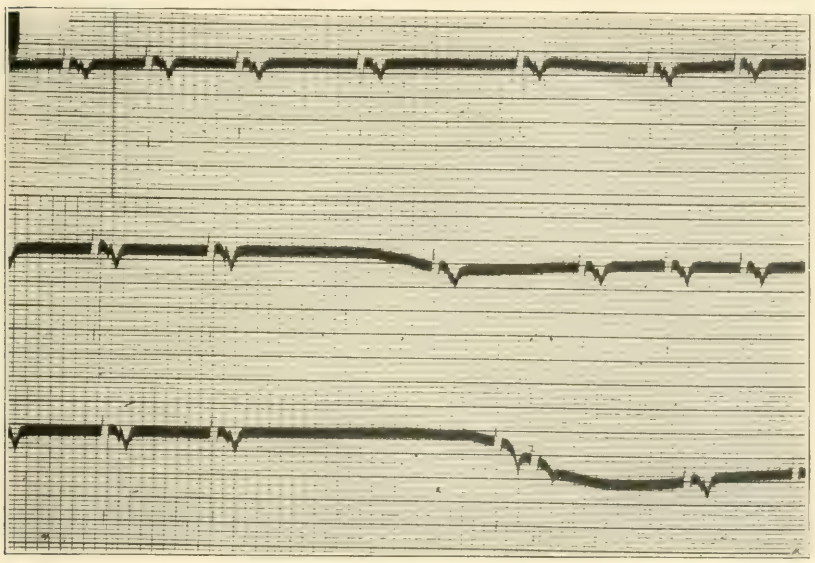


Fig. 18.—Lead III. Showing in lowest record a coupled beat induced by the lengthening of the *R-P* interval by right vagal pressure. Taken April 12, 1915.

one-tenth of a second, to 0.320 second; with the continuation of the more rapid heart rate, however, the auricular deflections disappeared (Figs. 14, 15, 16).

FURTHER OBSERVATIONS

Premature Beats.—On three occasions premature beats were recorded electrocardiographically (one shown in Fig. 19). Twice the premature contraction came at a time when a progressive slowing of the *a-v* rhythm was taking place; on the third occasion the beats prior to the premature beat were not recorded. The site of the impulse formation in the case of the heterogenetic contractions seems to have

been the same in all three, since the shape of the complexes is identical, and since the *P-R* interval is fairly constant (0.140± second). It is obvious that these premature beats arise from a point proximal to the location of the *a-v* pacemaker, probably at or near the auricular end of the *a-v* junctional tissues as suggested by the invert character of the auricular complex and by the relatively short *as-vs* interval. The pause following the premature beat is not compensatory; in one case this returning ventricular cycle is longer than the initial ventricular cycle by one-fifth of a second (Fig. 19); in a second instance they are equal, and on the third occasion there is no initial cycle for comparison, but the returning and first restored cycles are equal. In the

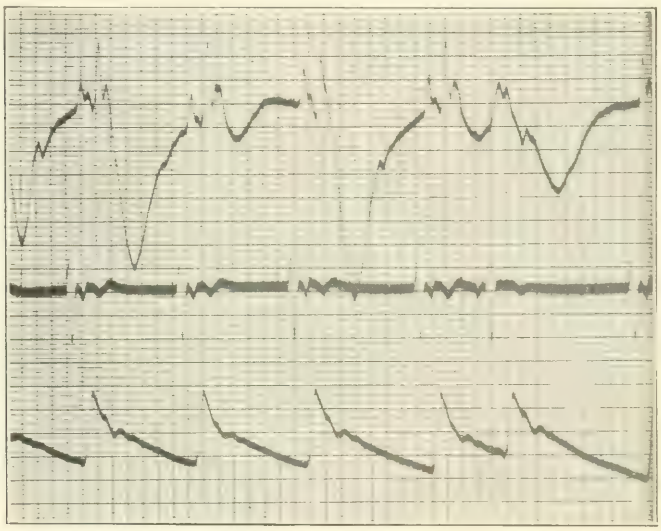


Fig. 19.—Polygram taken March 22, 1915, showing jugular pulse above, radial pulse below and Lead II of the electrocardiogram between. A premature beat interrupts the rhythm.

first two instances the *R-P* interval following the premature beat is of nearly the same length as are the *R-P* intervals preceding the premature beat (0.198 and 0.195 second as compared with 0.194 and 0.190 second, respectively).

Bigeminal Beats.—Aside from the premature beats mentioned above coupling of the contractions has sometimes occurred during the course of the *a-v* rhythm and only when there has been prolongation of the *R-P* interval through the action of digitalis or of vagal pressure. The coupling consists of a sandwiching of an auricular contraction between two ventricular beats (Figs. 14 and 18). In this sandwich the *P-R* interval has proved shorter than the *R-P* interval; for

example, 0.240 second as compared with 0.378 second in one of the digitalis couples and 0.289 second as compared with 0.376 second in one of the vagal pressure couples. The mechanism of production of these paired beats is not clear. Several explanations are possible. Premature beats may be arising in the *a-v* pacemaker and resulting in ventricular response, while the auricles are cut off by "backward block," the increasing depression of the auriculonodal junction affording too great a resistance to the passage of the premature excitatory process. Another possibility, but less likely than the preceding one, is that the first beat of the pair consists of ventricular complex alone, the auricle being blocked off, while the second beat results from a premature contraction starting in some point proximal to the *a-v* pacemaker and reaching the auricle before it reaches the ventricle. Finally we may adopt the tentative suggestion of Lewis[9]

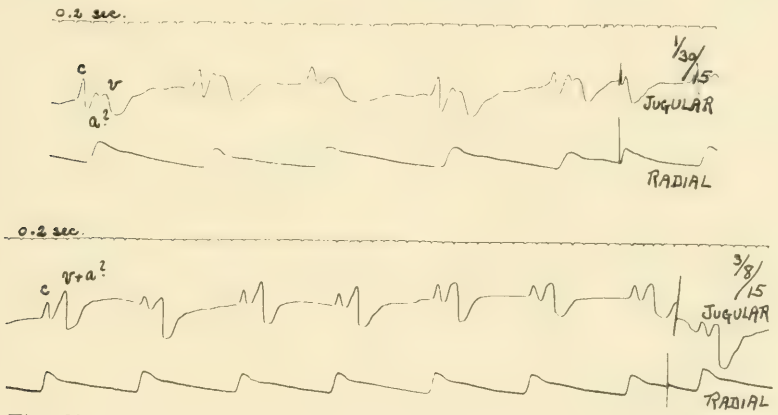


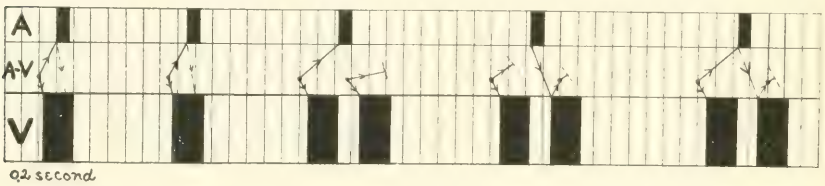
Fig. 20.—Polygrams showing jugular pulse above and radial pulse below. Taken January 30 and March 8, 1915. The auricular wave is apparently present preceding the *v* wave in the first record, and falling with the *v* wave in the second record.

that "the impulse center is not immediately on the path through which impulses pass from auricle to ventricle or ventricle to auricle; that it lies a little way off this channel and that a little time is lost while the impulse is traveling to it, though meanwhile the same impulse is passing on to stimulate the second main heart chamber." A diagram (Fig. 21) has been constructed to illustrate the probable mechanism of the *a-v* rhythm in the patient recorded here; the three pairs of beats represent the three methods in the production of the coupling in the order given above. In the last instance the delay in the passage of the excitatory process to the auricle allows the ventricle time to recover from its refractory period so that it may respond to

9. Lewis, Th., and White, P. D.: Heart, 1914, v, 359.

the new stimulus. In support of the third explanation are the facts that the coupling comes only when the *R-P* interval is very long (about 0.300 second or over), that paired beats ensue whenever this extreme prolongation occurs, and that coupling fails to follow when a beat appears without any auricular complex during the period of long *R-P* intervals.

Dislocation of the *a-v* pacemaker downwards through the action of digitalis or of vagal pressure would account for but a small part of the prolongation of the *R-P* interval.



Feb. 21.—Diagram illustrating the *a-v* rhythm of W. S. The first two beats represent the average rate and time relations of his rhythm. The three pairs of beats illustrate the possible mechanisms in the production of the bigeminy. The first couple shows a premature beat arising in the *a-v* pacemaker with the auricle blocked off; the second couple shows the blocking off of the auricle in the first beat of the pair, and immediately following occurs a premature beat near the auriculo-nodal junction; the third couple shows the ventricle responding first to the excitatory process coming from the *a-v* pacemaker and shortly after to an impulse coming down from the auriculonodal junction, which impulse starts downward as soon as the junction has been reached by the slowly traveling excitatory process originating in the side-tracked *a-v* pacemaker (ordinarily there is no response to this stimulus because the ventricle is still in the refractory stage when the process reaches it; the dotted lines in the first two beats of the diagram represent the excitatory wave reaching the ventricle while it is refractory). Abscissae = 0.2 second.

SUMMARY

1. A cardiac rhythm is described which arises in the atrioventricular junctional tissues of a patient who showed flutter and fibrillation of the auricles prior to the onset of the present rhythm.

2. This atrioventricular rhythm responds in rate readily to exercise, forced respiration, vagal pressure, and atropin, but not to digitalis.

3. The sequence of contraction *vs-as* is lengthened by vagal pressure and by digitalis, the latter apparently acting more on the auriculonodal junction than on the pacemaker in the *a-v* tissues.

4. Atropin may shorten the *vs-as* interval prolonged by digitalis, but with the uninfluenced *a-v* rhythm the effect of atropin on the *vs-as* interval is overborne by the increase in the rate of the heart beat resulting from the action of the drug on the *a-v* pacemaker; under such circumstances the *R-P* interval is increased slightly.

5. Premature beats interrupt the rhythm rarely; on such occasions the returning cycles are not compensatory.

6. Coupled beats of an unusual character appear when the *vs-as* interval is markedly prolonged by digitalis or by vagal pressure.

7. The constancy of the atrioventricular rhythm and the failure of sino-auricular rhythm to be restored by atropin or by vagal pressure point to a loss of function of the sino-auricular node, its task as pacemaker being assumed by the atrioventricular junctional tissues.

Thanks are due to Dr. E. W. Taylor for permission to study this case, to Mr. L. S. Brown and others who have been of assistance, and to the patient for his cooperation.

THE CREATININ OF THE BLOOD IN NEPHRITIS. ITS DIAGNOSTIC VALUE*

VICTOR C. MYERS, PH.D., AND WALTER G. LOUGH, M.D.
NEW YORK

The ability of the kidney to eliminate various substances administered either per os or parenterally has been practically employed by both the internist and surgeon as a means of determining its functional activity. The phenolsulphonephthalein test of Rowntree and Geraghty[1] and the lactose and potassium iodid tests of Schlayer and Takayasu[2] have found extensive use. More recently Neubauer[3] has suggested the administration of creatinin as a functional test, since it is the one normal urinary constituent which is entirely endogenous and the rate of excretion of added creatinin may, therefore, be readily followed.

The functional condition of the kidney may be ascertained by two quite different modes of attack: by the amount of certain substances excreted by the kidneys, or by their retention in the blood. Since the introduction during the past two years of simple methods of blood analyses, especially at the hands of Folin and his co-workers, the latter method has received considerable attention. Simple but relatively accurate methods for the nonprotein and urea nitrogen, uric acid, creatinin and creatin have thrown light on many disputed questions of abnormal metabolism, and further, have disclosed new possibilities in the way of practical diagnostic tests. The retention of the various nitrogenous waste products in certain forms of nephritis has long been known, although it has been only recently that we have had adequate data on the distribution of these compounds.

Normally, the nonprotein nitrogen amounts to 25 to 30 mg., the urea nitrogen to 12 to 15 mg., the uric acid to 1 to 2 mg., and the creatinin to 1 to 2 mg., all calculated per 100 c.c. of blood. As an illustration of the extent of retention, we have encountered figures of 350 mg. for the nonprotein nitrogen, 300 mg. for the urea nitrogen, 27 mg. for the uric acid and 33 mg. for the creatinin.[4] Both the urea

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* From the Laboratory of Pathological Chemistry and the Medical Service of the New York Post-Graduate Medical School and Hospital.

1. Rowntree, L. G., and Geraghty, J. T.: Jour. Pharm. and Exper. Therap., 1910, i, 579.
2. Schlayer and Takayasu: Deutsch. Arch. f. klin. Med., 1910-11, ci, 333.
3. Neubauer, O.: München. med. Wchnschr., 1914, lxi, 857.
4. Myers, V. C., and Fine, M. S.: Jour. Biol. Chem., 1915, xx, 391.

and nonprotein nitrogen have been extensively employed for diagnostic purposes.[5] In cases in which the nonprotein nitrogen and phenolsulphonephthalein tests have been simultaneously carried out, there has been a most excellent agreement between the two tests.[6] There is this difference, however; the amount of the phenolsulphonephthalein excretion shows the renal function at the moment, whereas the nonprotein and urea nitrogen are rather a measure between the amount of waste nitrogen produced in metabolism and that eliminated by the kidney. In the early stages of nephritis, the phenolsulphonephthalein test probably yields the more valuable information, but after a decided retention has taken place, the nonprotein and urea nitrogen of the blood furnish a much more accurate index of the changes in the condition of the patient. With nonprotein nitrogen values over 60 mg. and urea nitrogen values over 40 mg., the prognosis is bad. The dietetic treatment of nephritis based on the degree of nitrogen retention has likewise received favorable consideration.[7]

Ambard[8] and other French workers have claimed that the relation between the concentration of urea in the blood and that eliminated in the urine furnishes a more reliable index of the functional condition of the kidneys than the content of the urea in the blood alone. Since the experiments of the French workers were conducted with unreliable methods, they have attracted comparatively little attention in this country. The very recent experiments of Marshall and Davis,[9] and McLean and Selling[10] with reliable methods appear, however, to support their contention.

As would seem probable, nephritis with retention is accompanied not only by an accumulation of urea in the body, but by the other nitrogenous waste products as well, especially uric acid and creatinin. This has been amply demonstrated by the observations of Folin and Denis, and Myers and Fine, who noted very high values for both uric

5. Folin, O., and Denis, W.: Jour. Biol. Chem., 1913, xiv, 29; 1914, xvii, 487. Foster, N. B.: The Archives Int. Med., 1912, x, 414; 1915, xv, 356. Agnew, J. H.: ibid., 1914, xiii, 485. Frothingham, C., Jr., and Smillie, W. G.: ibid., 1914, xiv, 541. Tileston, W., and Comfort, C. W., Jr.: ibid., 1914, xix, 620. Farr, C. B., and Austin, J. H.: Jour. Exper. Med., 1913, xviii, 228.

6. Frothingham, C., Jr., Fitz, R., Folin, O., and Denis, W.: The Archives Int. Med., 1913, xii, 245. Frothingham and Smillie: ibid., 1914, xiv, 541.

7. Folin, O., Denis, W., and Seymour, M.: The Nonprotein Nitrogenous Constitutents of the Blood in Chronic Vascular Nephritis (Arteriosclerosis) as Influenced by the Level of Protein Metabolism, The Archives Int. Med., 1914, xiii, 224. Frothingham, C., Jr., and Smillie, W. G.: A Study of Different Nitrogenous Diets in Chronic Nephritis, ibid., 1915, xv, 204.

8. Ambard, L.: Compt. rend. soc. de biol., 1908, lxv, 712; 1910, lxix, 411 and 506.

9. Marshall, E. K., Jr., and Davis, D. M.: Jour. Biol. Chem., 1914, xviii, 53.

10. McLean, F. C., and Selling, L.: Jour. Biol. Chem., 1914, xix, 31.

acid and creatinin in the severer cases of interstitial nephritis. Since atophan rapidly reduces the uric acid concentration of the blood in normal and gouty individuals,[11] but is without influence in the severer cases of nephritis with marked retention, Fine and Chace[12] have suggested its use as a means of determining the degree of renal involvement.

The marked retention of creatinin in the blood in uremia was noted independently and almost simultaneously by three different groups of investigators, Neubauer,[13] Folin and Denis,[14] and Myers and Fine.[15] More detailed observations have since been presented by Myers and Fine[16] while Foster[17] has reported a few analyses of the total creatinin (creatinin plus creatin). It has long been known that in severe nephritis creatinin was generally excreted in decreased amounts,[18] and it was quite logical to conclude from this that the permeability of the kidney to creatinin had been decreased. That such is the case is evident from the data at hand.

The present communication concerns itself primarily with the retention of creatinin in the blood, and since this occurs, practically speaking, only in nephritis, discussion will largely be confined to this condition. Although this retention of creatinin in uremia has been recognized now for more than a year, the subject has not received attention from a clinical point of view. In our hands the estimation of the blood creatinin has been of considerable prognostic value, cases showing over 5 mg. per 100 c.c. of blood having invariably terminated fatally. Table 1 gives the results obtained in thirty cases of nephritis. To give a more comprehensive picture of the cases, the observations for the urea and uric acid of the blood, the blood pressure, the phenolsulphonephthalein output and the urinary findings have been included as well as the creatinin of the blood. The results of the blood analyses given in this table are those obtained at the height of the retention. The blood analyses of some of the older cases were reported in a previous paper,[16] but are included here since the clinical side of the problem was not considered. For other and more detailed analyses on

11. Fine, M. S., and Chace, A. F.: Jour. Pharm. and Exper. Therap., 1914, vi, 219.

12. Fine and Chace: THE ARCHIVES INT. MED. To be published.

13. Neubauer, O.: München. med. Wchnschr., 1914, lxi, 857.

14. Folin, O., and Denis W.: Jour. Biol. Chem., 1914, xvii, 487.

15. Myers, V. C., and Fine, M. S.: Proc. Soc. Exper. Biol. and Med., 1914, xi, 132. Myers: Post-Graduate, 1914, xxix, 429.

16. Myers and Fine: Jour. Biol. Chem., 1915, xx, 391.

17. Foster, N. B.: THE ARCHIVES INT. MED., 1915, xv, 356.

18. Ditman, N. E., and Welker, W. H.: New York Med. Jour., 1909, lxxxix, 100, 1046, 1091 and 1134. Also Kraus, W. M.: THE ARCHIVES INT. MED., 1913, xi, 613.

these cases reference may be made to this paper. Table 2 gives the figures for the creatinin, urea and uric acid of the blood at different intervals in seven of our recent cases of nephritis illustrating varying degrees of severity. Figures for the creatinin of the blood in a large number of miscellaneous conditions are given in Table 3 with the view of showing that it is only in nephritis that high values for creatinin are encountered. Figures for the creatin of the blood are not included in the present paper because they are not regarded as significant in this connection. The question of the creatin of the blood will be discussed in another paper dealing with the urinary excretion of creatin.

METHODS EMPLOYED

The phenolsulphonephthalein was administered intramuscularly according to the method of Rowntree and Geraghty[19] and the excretion estimated with the Hellige colorimeter. The methods which we have employed in the analysis of blood have already been described.[20] It may be well, however, to outline the technic which has been used for the estimation of the creatinin.

TECHNIC

In most of our work the Duboscq colorimeter has been utilized for the estimation of the creatinin and creatin somewhat after the technic described by Folin.[21] About 10 c.c. of blood are drawn directly from a vein into a small bottle containing a little powdered potassium oxalate or 5 drops of a 20 per cent. solution to prevent clotting. Six c.c. of the well mixed blood are treated with 24 c.c. of water (4 volumes) in a 50 c.c. centrifuge tube. After the corpuscles have been laked, about 1 gram of dry picric acid is added, and the mixture stirred at intervals with a glass rod until it is a light yellow. When the protein precipitation is complete, the tube is centrifuged and the supernatant fluid filtered through a small 7 cm. filter paper. From 17 to 21 c.c. of filtrate are usually obtained (5 c.c. of which are removed for the creatin estimation and 3 c.c. for the sugar estimation, if these determinations are to be carried out). To 10 c.c. of the filtrate is added 0.5 c.c. of 10 per cent. sodium hydroxid, and a similar amount of alkali added to 10 c.c. of standard creatinin in saturated picric acid (containing 0.2, 0.5 or 1.0 mg. creatinin to 100 c.c. of picric acid). Creatinin may now readily be prepared perfectly pure by the admirable method of Benedict.[22] A standard solution of this creatinin, 1 mg. to 1 c.c., is kept in 0.1 N hydrochloric acid. From this we have prepared a stock solution in picric acid, 5 mg. to 100 c.c., by diluting 5 c.c. to 100 c.c. with saturated picric acid. By pipetting 0.4, 1.0 and 2.0 c.c. of this solution into 10 c.c. graduates with a Mohr pipet and diluting to the mark, standards of the above strengths are prepared. For the Duboscq colorimeter the standard prism can conveniently be set at the 15 mm. mark.

19. Rowntree, L. G., and Geraghty, J. T.: The Archives Int. Med., 1912, ix, 284.

20. Myers, V. C., and Fine M. S., with the collaboration of Bailey, C. V., and Gorham, F. D.: Chemical Composition of the Blood in Health and Disease, a series of papers reprinted from the Post-Graduate for 1914-15.

21. Folin, O.: Jour. Biol. Chem., 1914, xvii, 457.

22. Benedict, S. R.: Jour. Biol. Chem., 1914, xxiii, 183.

TABLE 1.—Creatinin Content of the Blood in Nephritis

Case	Age	Sex	Type of Nephritis	Result	Blood Analysis			Phthalein, 2-Hour Output	Blood Pressure		Urine		Remarks
					Creatinin, Mg. to 100 c.c.	Urea N, Mg. to 100 c.c.	Uric Acid, Mg. to 100 c.c.		Systolic	Diastolic	Albumin	Casts	
1 W. F...	25	♂	Mercury poisoning, uremia	Died.....	33.3	240	15.0	....	155	70	++	++	Uremic symptoms appeared one week previous to death although the creatinin had dropped to 14.8 mg. Died Dec. 5, 1914
2 T. D....	34	♂	Chronic interstitial, uremia	Died.....	20.5	152	14.3	2-3	230	130	++	+++	Dizziness, retinal hemorrhages. Died March 23, 1915
3 I. D....	17	♀	Chronic interstitial, uremia	Died.....	20.0	209	27.0	0	165	115	++	++	Marked pyorrhea alveolaris; moderate dilatation of heart. Died Jan. 4, 1915
4 E. C...	50	♀	Chronic interstitial, uremia	Died.....	16.7	236	22.4	0	210	100	++	.....	Died Jan. 11, 1915, two days after admission
5 W. O'C.	33	♂	Chronic interstitial, uremia	Died.....	16.6	182	11.4	1	213	...	++	+	Slight edema of legs and ankles at time of admission; albuminuric retinitis; marked cyanosis and dyspnea. Died May 10, 1914
6 M. K...	42	♂	Chronic diffuse, uremia	Died.....	14.7	170	14.0	....	...	...	++	++	Moderate hypertrophy of heart; marked anemia and cyanosis; coma. Both kidneys decapsulated on May 13, 1914. Patient died during operation.
7 K. K....	39	♀	Chronic interstitial, uremia	Died.....	14.7	148	12.5	0-0	204	108	+++	.....	Considerable cardiac hypertrophy; accentuation of second aortic sound. Died March 27, 1915
8 J. W...	34	♂	Chronic interstitial, uremia	Died.....	11.0	144	8.7	3-4	190	120	++	+	Occasional edema of ankles previous to admission; slight cardiac hypertrophy; marked neuroretinitis. Died April 16, 1915
9 J. S....	50	♂	Chronic diffuse, uremia	Died.....	7.4	81	13.4	....	112	88	++	++	Slight edema of lower extremities; suppression of urine. Died Nov. 19, 1914
10 W. G...	8	♂	Chronic diffuse, edema, uremia	Died.....	7.0	91	5.5	3-8	153	105	++++	+++	Purpura hemorrhagica; marked general anasarca; died after being removed from hospital against advice
11 E. E....	30	♀	Chronic interstitial, edema, uremia	Died.....	5.3	100	15.4	10	225	160	++	+++	Wassermann ++++, generalized edema. Died Jan. 19, 1915
12 L. P....	57	♂	Chronic interstitial, uremia	Improved..	4.8	80	8.0	1-31-10	295	145	++	+	Marked edema of feet and ankles previous to admission; thickening of arterial walls; left hospital improved
13 L. R....	52	♂	Chronic interstitial	Improved..	4.6	40	5.5	47	167	98	++	++	Edema of ankles; marked accentuation of second aortic sound; left hospital Jan. 24, 1915

14 L. R....	52	♀	Chronic interstitial	Improved..	3.3	17	4.1	16	180	94	+	+	Epigastric pain; vomiting; enlarged liver; paroxysmal tachycardia; slight edema of feet and ankles; left hospital Dec. 12, 1914
15 J. P....	34	♂	Chronic diffuse.....	Improved..	3.2	72	8.3	25-15-43	235	130	++	+++	Wassermann ++++; generalized edema; still in hospital
16 L. B....	64	♀	Chronic interstitial	Improved..	3.1	33	2.2	56	252	125	+	+	Slight edema of feet and ankles; slight cardiac hypertrophy; left hospital improved, Jan. 24, 1915
17 L. S....	23	♀	Chronic parenchymatous	Improved..	3.1	18	4.6	31-33	116	75	++	+	Slight edema of feet and ankles; enlarged tonsils; teeth in poor condition; left hospital improved.
18 C. M....	55	♂	Chronic diffuse.....	Improved..	2.9	16	3.6	29-43	210	125	++	+++	Generalized edema and ascites; glycosuria and hyperglycemia (0.20 per cent.); improving
19 J. M....	50	♂	Chronic interstitial	Improved..	2.8	18	6.6	22	196	144	++	++	Arteriosclerosis; left hospital improved
20 H. B....	59	♀	Chronic interstitial	Slightly improved	2.8	17	5.9	2-8-39	145	90	++	+++	Patient does not appear ill despite the low phthalein output
21 F. G....	20	♀	Pyonephrosis.....	Unchanged	2.7	24	....	25	110	55	++	.....	Left hospital against the advice of physician
22 F. S....	51	♀	Chronic interstitial	Unchanged	2.6	14	2.9	42	150	80	+	++	Cholelithiasis; cholecystitis; patient still in hospital
23 A. S....	44	♀	Chronic interstitial	Improved..	2.5	27	....	74	216	140	++	++	Patient appeared to show some uremic symptoms on admission
24 L. S....	55	♂	Chronic interstitial	Unchanged	2.1	29	6.3	....	175	65	+	+	Wassermann ++++; spinal fluid ++++; aortic insufficiency; left hospital improved
25 W. McL.	42	♂	Chronic interstitial	Improved..	2.1	20	4.0	34	165	110	+	+	Epistaxis; headaches; anemia; left hospital improved
26 F. B....	60	♂	Chronic diffuse.....	Died.....	1.8	29	4.5	33	170	115	++	+++	Marked dyspnea; fluid in abdomen. Died on June 16, 1914
27 H. S....	37	♀	Chronic interstitial	Died.....	1.8	25	2.9	17-3-12	143	...	++	+++	Generalized edema; double decapsulation. Died March 27, 1914
28 F. S....	67	♂	Chronic interstitial	Unchanged	1.5	10	4.2	22-10	114	77	+	+	Arteriosclerosis; cystitis
29 H. K...	26	♂	Acute parenchymatous, edema	Improved..	0.9	15	2.6	29-32	125	72	+++	+++	Double decapsulation
30 A. K....	52	♀	Chronic interstitial	Improved..	0.9	28	3.6	39	182	105	+	+	Physical examination negative

TABLE 2.—Progressive Changes in the Creatinin, Urea and Uric Acid of the Blood in Typical Cases of Nephritis

Case	Date	Blood Analyses			Remarks
		Creatinin, Mg. to 100 c.c.	Urea N, Mg. to 100 c.c.	Uric Acid, Mg. to 100 c.c.	
7 K. K.	3/20/15	9.4	110	10.9	
	3/24/15	9.7	138	12.4	
	3/26/15	14.7	148	12.5	Patients showed definite uremic symptoms on admission and rapidly went into coma and died
4 E. C.	4/ 9/15	11.5	90	15.9	
	4/11/15	16.7	236	22.4	
2 T. D.	1/22/15	7.8	60	7.0	
	1/27/15	10.0	67	7.0	
	1/30/15	8.9	77	6.8	
	2/ 6/15	8.4	72	6.0	
	3/ 9/15	11.0	97	9.1	
	3/16/15	11.4	120	10.8	
	3/19/15	14.1	131	10.5	
	3/23/15	20.5	152	14.3	Patients on admission in fair condition clinically and the approach of uremia did not appear evident. Both cases showed slight improvement on treatment, although Case 2 was evidently more severe than Case 8. With T. D. uremic symptoms appeared about two weeks before death, whereas with J. W. they were not evident until four days previous to death
8 J. W.	2/24/15	8.3	55	8.1	
	3/ 1/15	6.5	63	5.6	
	3/ 6/15	5.3	89	6.0	
	3/19/15	6.5	51	4.2	
	3/25/15	6.1	51	7.0	
	4/ 8/15	8.0	61	7.2	
	4/15/15	11.0	144	8.7	
12 L. P.	1/ 6/15	4.8	180	8.0	Case 12 showed severe uremic symptoms on admission, but readily responded to treatment and left the hospital improved
	1/22/15	3.6	65	2.2	
	2/18/15	3.1	59	3.6	
	3/ 1/15	2.9	37	4.9	
	3/ 5/15	2.5	45	2.5	
15 J. P.	4/23/15	3.2	72	8.3	Patient on admission showed some dyspnea and general anasarca, but readily responded to treatment
	5/ 4/15	2.4	37	6.7	
	5/12/15	2.3	28	5.7	
18 C. M.	4/15/15	2.3	15	5.4	Patient entered hospital with great respiratory distress due to marked ascites. After abdominal paracentesis, symptoms were greatly relieved and the condition much improved. Blood sugar of 0.20 per cent.
	4/27/15	2.9	16	3.6	

TABLE 3.—Creatinin Content of the Blood in Miscellaneous Conditions

Case	Clinical Diagnosis	Creatinin of Blood, Mg. to 100 c.c.	Case	Clinical Diagnosis	Creatinin of Blood, Mg. to 100 c.c.
31 C. ...		1.3	64 H. H.	Acromegaly.....	1.0
32 H. R.		0.8	65 S.	Amputation diabetic gangrene(?)	1.8
33 B. ...		1.3	66 A. F.		1.5 (2)
34 S. ...	Normal.....	1.6	67 M. G.		1.4
35 J. F.		0.9	68 P. F.		1.7
36 C. C.		1.2	69 J. E.		1.5
37 J. B.		1.9	70 M.Mc.	Diabetes.....	1.1-3.8 Av. 2.3 (6)
38 E. B.	Malnutrition.....	1.3	71 C. L.		0.9
39 I. S.		1.3	72 F. R.		1.4-2.1 Av. 1.7 (2)
40 S. ...		1.5	73 R. J.		1.9-3.0 Av. 2.5 (2)
41 M. F.		1.9	74 M. W.		2.0
42 M. S.		2.9	75 M.Mc.		1.5-2.6 Av. 2.0 (2)
43 F. S.	Chronic interstitial nephritis	2.8	76 M. W.		2.2
44 A. A.		1.0-2.4 Av. 1.7 (2)	77 A. B.		2.6
45 Dr. M.		2.2	78 C. N.	Diabetes (blood did not show hyperglycemia)	2.3
46 R. C.		1.1	79 J. G.		1.0
47 F. S.	Acute parenchymatous nephritis	2.6	80 B. ...		1.3
48 S. R.	Nephroptosis.....	1.2	81 L. J.		2.2
48 E. G.	Endocarditis.....	2.8	82 P.O'N.		0.8-1.5 Av. 1.1 (4)
50 F. ...	Carcinoma.....	3.1	83 T. B.	Gout.....	0.9-2.2 Av. 1.4 (8)
51 H. S.	Carcinoma of bladder	1.8	84 J. L.		1.7
52 J. L.	Carcinoma.....	1.4	85 W. L.		2.5
53 S.	Brain tumor.....	2.0	86 J. H.		0.8
54 J. K.	Hyperthyroidism	3.1	87 M. B.		1.1-2.2 Av. 1.5 (3)
55 B. S.	Exophthalmic goiter	2.2	88 M. L.		1.5-2.5 Av. 2.0 (2)
56 J. E.	Leukemia.....	1.3-2.8 Av. 2.2 (2)	89 J. S.	Arthritis.....	2.1
57 T.v.S.	Pernicious anemia	1.4-2.3 Av. 1.8 (2)	90 Dr. S.		2.2
58 M. S.	Secondary anemia	0.8	91 M. A.		1.4-2.2 Av. 1.8 (2)
59 H. B.	Secondary anemia with hemorrhoids	1.2	92 A. O.		2.6
60 J. B.	Hemolytic jaundice	2.2	93 A. K.	Acute articular rheumatism	2.1
61 B. W.	Bronchial asthma	2.3	94 A. G.	Pleurisy with effusion	2.2
62 F. ...	Neurasthenia.....	1.6	95 J. M.	Gumma of liver	1.0
63 L. ...	Cholelithiasis.....	2.1	96 L. M.	Carcinoma of liver	1.1

Notes on Cases With High Creatinin Values

42. Phthalein 35 per cent.; many casts, but no albumin.
43. Phthalein 30 per cent.; trace of albumin.
47. Phthalein 39 per cent.; moderate amount of albumin and moderate number of casts.
49. Phthalein 75 per cent.; few casts, but no albumin.
50. Phthalein 52 per cent.; Wassermann, one plus; moderate amount of albumin and many red blood cells, systolic blood pressure 165.
54. Heart-block, 0.5 per cent. sugar in urine.
70. Phthalein 52 per cent., trace of albumin.
73. Phthalein 36 per cent., moderate amount of albumin.
75. Phthalein 71 per cent., moderate amount of albumin and moderate number of casts.
77. Gastric ulcer; trace of albumin.
92. Phthalein 58 per cent.: moderate amount of albumin; few casts.

The estimation of the creatinin may likewise be carried out with the use of the Autenrieth-Königsberger colorimeter of Hellige. In this case less blood is necessary. Two c.c. are treated in a cylindrical centrifuge tube with 8 c.c. of water and other manipulations as above. For the determination proper, 0.1 c.c. of 10 per cent. sodium hydroxid are added to 2 c.c. of the picric acid filtrate in a small test tube. Simultaneously 1 c.c. of the alkali is added to 20 c.c. of a saturated solution of picric acid containing 1.5 mg. creatinin to 100 c.c. to serve as standard for the wedge. At the end of ten minutes the wedge is filled with the standard, the cup with the unknown, and readings made. Although it is well to calibrate a wedge for a given instrument, the following formula, in which R represents the colorimetric reading and 5 the dilution, will suffice:

$$89 - R \times 0.0179 \times 5 = \text{mg. creatinin calculated per 100 c.c. of blood.}$$

DISCUSSION

Of the eleven cases in our series[23] (Table 1) showing over 5 mg. of creatinin per 100 c.c. of blood, all terminated fatally in from a few days to two months. In this group of cases the phthalein output was practically zero, with the exception of Case 11, and here the test was carried out over two months before death. It should perhaps be noted that Case 20 on the first two tests showed very low phthalein outputs, although the creatinin of the blood was only slightly raised. Cases 12 and 15 with very high figures for urea nitrogen (80 and 72 mg.) and uric acid (8.0 and 8.3 mg.), but with comparatively low values for creatinin (4.8 and 3.2 mg.), showed considerable improvement under treatment.

From the distribution of the nonprotein nitrogenous compounds in the blood and urine, it would appear that of the three constituents mentioned, creatinin was normally the most readily, and uric acid the least readily, eliminated, with urea standing in an intermediate position (see Table 4). From this it is logical to expect that the excretion of uric acid would be the first to be impaired. In gout this is apparently the only constituent for which the permeability is lowered, while in nephritis our blood studies appear to indicate that a retention of uric acid is generally the first to become evident. In harmony with this hypothesis we should next expect a retention of urea, and lastly that of creatinin. This appears to correspond fairly well with the facts in most cases. It seems reasonable to conclude that when a noticeable retention of creatinin has occurred, the functional condition of the kidney has been gravely impaired. In our experience creatinin values from 2.5 to 3.0 mg. per 100 c.c. of blood should be viewed with suspicion and figures of from 3.0 to 5.0 mg. regarded as decidedly unfavorable, while figures over 5 mg. probably indicate an early fatal termination.

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23. For the opportunity of studying these cases we are under obligation to the Director of the Medical Department, Dr. Edward Quintard. We are also indebted to Drs. A. F. Chace and R. A. Cooke and other members of the medical staff for many courtesies extended to us, and to Dr. M. S. Fine of the Laboratory of Pathological Chemistry for the uric acid estimations he has collected on this series of cases.

Theoretically the amount of the increase in the creatinin of the blood should be a safer index of the decrease in the permeability of the kidney than either urea or uric acid, for the reason that creatinin on a meat-free diet is entirely endogenous in origin and its formation (and elimination normally) very constant. Urea, on the other hand, is largely exogenous under normal conditions and its formation, therefore, subject to great fluctuations, the same being true in a measure of uric acid.

Of the data given in Table 2, the creatinin figures indicate especially well the condition of the patients at the time the examinations were made. The values found for the creatinin in Case 12 dropped gradually with the improvement in the case, but an inspection of the table does not show the same regularity in the decline of the urea and uric acid. The same remarks might be made in a general way for Cases 2 and 8. With the improvement there was a decline in the creatinin, while with the relapse the values soon passed their former maximum and gradually rose until the fatal termination. As would seem evident from this table, the blood analyses are much more valuable than the phthalein in showing slight changes in severe cases of nephritis.

TABLE 4.—COMPARATIVE NITROGEN PARTITION OF URINE AND BLOOD IN PER CENT. OF TOTAL NONPROTEIN NITROGEN

Fluid	Urea N	Uric Acid N	Creatinin N	Ammonia N	Undetermined N
Normal urine	85	2	5	4	4
Normal blood	50	2	2	0.3	46
Blood in severe uremia...	75	2.4	2.5	0.5	20
Blood in chronic nephritis	55	2.2	2	0.3	41
Blood in gout.....	50	6.0	2	0.3	42

We have taken occasion to estimate the creatinin content of blood in a large number of miscellaneous conditions, the results of which are given in Table 3. In eleven of these cases figures between 2.6 and 3.1 were found. Three of these had been diagnosed as nephritis but this was not true of the remaining eight. An inspection of the histories of these cases (diabetes, arthritis, carcinoma, endocarditis), however, showed almost invariably evidence of some renal involvement. It is of interest that in gout with its uric acid retention there appears to be no appreciable retention of creatinin. It seems safe to conclude that the creatinin of the blood is raised only in conditions resulting in a decreased activity on the part of the kidney.

There seems to be no evidence, as already pointed out,[16] which definitely supports the view that the retention of metabolic end products such as urea, uric acid and creatinin, are themselves the cause of uremic symptoms. In Case 1, of mercury poisoning, uremic symptoms did not appear until a week after maximum concentrations had been attained. The possibility that a decomposition product of creatinin[18] such as methylguanidine, might play a part in the development of uremia, should perhaps still be considered, although we now possess other explanations for the onset of this condition.[24]

CONCLUSIONS

The estimation of the creatinin of the blood in nephritis is a very simple but valuable diagnostic and prognostic test. The creatinin rises above 2.5 mg. per 100 c.c. of blood almost without exception only in conditions with renal involvement. Creatinin values from 2.5 to 3.0 mg. may be viewed with suspicion; figures from 3.0 to 5.0 mg. regarded as decidedly unfavorable, while over 5.0 mg. probably indicate an early fatal termination.

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24. Peabody, F. W.: The Archives Int. Med., 1914, xiv, 236.

A METHOD FOR THE DETERMINATION OF PLASMA AND BLOOD VOLUME*

N. M. KEITH, M.D., L. G. ROWNTREE, M.D.
AND
J. T. GERAGHTY, M.D.
BALTIMORE

Routine red cell counts and hemoglobin determinations yield information that is valuable but very incomplete. This information concerns only the concentration of the blood. These routine methods do not furnish us absolute values for hemoglobin, for red cells, or for the oxygen-carrying capacity of the blood. With them, an anemia may be more apparent than real, as it may be dependent on or associated with a marked increase in plasma volume, while a polycythemia does not necessarily indicate an absolute increase in red cells, as it may be dependent on a decrease in plasma. In order to obtain absolute values for either red cells or hemoglobin, data relative to plasma volume or total blood volume are essential.

The volume as well as the concentration of the blood must play a rôle in pathological physiology. Absolute values are essential to the proper interpretation of certain pathological findings. Is an increase in the blood mass with overfilling of the vascular system in any way responsible for hypertension? Is the large heart frequently seen in pernicious anemia due to a large blood volume? Is the large heart sometimes seen in myocardial insufficiency (unassociated with hypertension or nephritis) due to an increase in blood mass? Questions such as these must remain unanswered until data concerning absolute values are correlated with pathological findings.

With so great variations in size and weight of individuals, the normal values for plasma and blood are best expressed in percentages or fractions of the body weight, or as the number of cubic centimeters per kilogram of body weight. Since the work of Welcker, the blood has been considered to constitute one-thirteenth of the body weight. Recently, the values obtained by Haldane and Smith (one-twenty-first), and by Plesch (one-nineteenth) have been frequently accepted. The methods affording these last values are not without serious drawbacks from the points of view of inherent technical error, complicated nature of apparatus required and the possibility of injury or discomfort attending their use.

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* From the Medical and James Buchanan Brady Urological Clinics of the Johns Hopkins Hospital.

There is a distinct need for a simple method for the determination of plasma and blood volume, particularly for a method that can be repeatedly applied without injury to the patient.

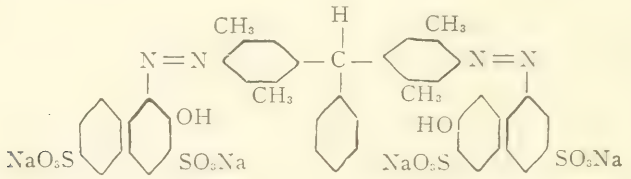
THE PRINCIPLE OF THE METHOD

The principle underlying this method is the introduction directly into the circulation of a non-toxic, slowly absorbable dye, which remains in the plasma long enough for thorough mixing, and the determination of its concentration in the plasma colorimetrically by comparison with a suitable standard mixture of dye and serum.

A dye which would fulfil these conditions was sought. Certain of the phthaleins were tried and discarded because of the rapidity with which they leave the vascular system. Dr. Herbert M. Evans, of the Anatomical Department, when consulted in regard to dyes remaining for a long time in the circulation, suggested the possibilities of vital red, and supplied us with the dye used in this work. It is with great pleasure that we acknowledge our indebtedness to him.

SOME PROPERTIES OF THE DYE

Vital red is disodium disulphonaphthol azotetramethyl triphenyl methane.



It is water soluble to the extent of 1½ per cent. In dilute solutions of 0.01 per cent., it does not dialyze against water through collodion sacs even in forty-eight hours. Under such conditions, the dye is practically adsorbed by the dialyzing membrane.

When its toxicity was determined by intravenous injection into animals, it was found that doses exceeding from 5 to 10 mg. per kilo sometimes resulted in a slight transient albuminuria. Smaller doses were harmless, so far as could be ascertained. Three mg. per kilo was chosen as the most suitable amount for our purposes. Protocols of cats and dogs studied for toxicity appear below.

TOXICITY EXPERIMENTS

PROTOCOLS

CAT 1.—Weight, 2.4 kg.; given 750 mg. intravenously; killed in three hours.

CAT 2.—Weight, 2.75 kg.; given 700 mg. intravenously; killed in twenty hours.

CAT 3.—Weight, 1.75 kg.; given 6 mg. to kilogram intravenously; no effect; urine free from albumin afterward.

Dog 5.—Weight, 7 kg. Nov. 4, 1914: Given 600 mg.; vitally stained; no toxic manifestations. One week later, 40 per cent. of dye still in circulation.

Dec. 17, 1914: Blood still contains considerable dye.

March 1, 1915: Animal still slightly vitally stained after a period of four months. On only one occasion during this whole period was a trace of albumin found in the urine.

Dog 8.—Weight, 6.85 kg. Nov. 17, 1914: Given 350 mg.

Nov. 18, 1914: Considerable albumin in urine; no casts; some red blood cells.

Nov. 22, 1914: Advanced distemper. Urine free from albumin.

Nov. 26, 1914: Died with distemper.

Dog 9.—Weight, 9.9 kg. Nov. 21, 1914: Given 99 mg. Dog was normal except for a faint trace of albumin found in the urine on first and second days.

Dog 10.—Weight, 5.5 kg. Nov. 21, 1914: Given 55 mg.; normal; no albumin.

In order to vitally stain the tissues even slightly, it was found necessary to use at least 10 mg. per kilo. If a strong solution is injected locally into the subcutaneous tissues, the tissues are stained a beautiful bright red color, which persists unaltered for many months. This accident happened with patients on two or three occasions early in this work, but since has been avoided by ascertaining with certainty that the needle was in the lumen of the vein before the dye was injected.

Traces of the dye appear in the urine and in the lymph flowing from the thoracic duct at the end of ten minutes. The dye continues to be excreted in the urine of patients for from three to four days following a dose of 3 mg. per kg. Following this amount no evidence of the dye in the tissue cells or white blood cells could be found with the oil immersion. The fate of all the dye has not been determined, but considerable amounts can be found in the plasma for three or four days following its injection.

TECHNIC

A 1.5 per cent. solution of the dye in freshly distilled water is prepared and sterilized by boiling for from five to ten minutes, and aseptically stoppered in a sterile Erlenmeyer flask. This solution is made up as required.

The patient is weighed (stripped) and the dose determined on a 3 mg. per kilo basis. The skin over the anterior surface of both elbows is cleaned up in the usual manner for venepuncture. Under aseptic technic, a small platinum-iridium needle (20 gauge) to which is attached a short piece of rubber tubing (from 2 to 3 cm. in length) is inserted into the vein. Ten c.c. of blood are withdrawn into a pipet and emptied into a 50 c.c. centrifuge tube containing just sufficient powdered sodium oxalate to prevent coagulation. The plasma so obtained is used in the preparation of the standard which is described

later.[1] The rubber tubing is removed from the needle, the latter being left in the vein and a 20 c.c. Record syringe containing the exact dose[2] of the dye is attached, and its contents injected, after which the needle is withdrawn. Three minutes later, a needle is inserted into the vein of the opposite arm. Without impeding the venous return, 10 c.c. of blood are withdrawn immediately by means of a pipet; two or three minutes later, 10 c.c. more are withdrawn. The first is placed in a 50 c.c. centrifuge tube, the second in a 15 c.c. graduated centrifuge tube. To each, sufficient dry oxalate is added to prevent clotting, and the tubes are capped with rubber to prevent evaporation during centrifugalization.

The three tubes are placed in a high-power centrifuge and subjected to 3,000 revolutions per minute for twenty minutes.[3] After removal from the centrifuge, the relative proportions of erythrocytes and plasma in the graduated tube are noted. Without disturbing the red cells, the plasma is carefully pipetted off from each tube and placed in a clean test tube. We now have three test tubes, (1) containing plasma without dye; (2 and 3) containing plasma with dye in an unknown concentration. A suitable dye-plasma standard mixture of known dye content must be prepared, and with it 2 and 3 are compared.

The standard dye preparation[4] is made as follows: 0.5 c.c. of the dye solution used for the injection is brought up accurately to 100 c.c.

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1. This procedure of taking plasma for mixing the standard in each case was found necessary on account of the great variation in the color of the plasma in different individuals.

2. The amount injected is 3 mg. per kg. The weight in pounds divided by eleven gives the number of cubic centimeters of a 1.5 per cent. solution to be injected.

3. To prevent hemolysis, all pipets and centrifuge tubes must be thoroughly washed with normal saline solution and dried before use. It is obvious that the slightest hemolysis will alter the color of the plasma and make accurate colorimetirc readings impossible.

4. Obviously the standard must be sufficiently intense in color to insure the patient's readings falling on the scale. Since the smaller the amount of plasma in relation to the body weight the greater will be the intensity of the dye, a standard must be chosen which will be more intense than the plasma-dye mixture obtained from normal patients having the smallest proportion of plasma in relation to body weight. The standard selected has been prepared on the following basis: The amount of drug injected is placed in the number of cubic centimeters of salt solution corresponding to the number of grams represented by 4 per cent, of the body weight, or, expressed differently, in the amount of salt solution represented by 40 c.c. for every kilogram of body weight; this amounts to 5 c.c. (7.5 mg. of dye) of the solution injected, to 100 c.c. of 0.8 per cent. sodium chlorid. For example, a man weighing 70 kilos would be given 210 mg. of dye (3 mg. per kg.), which is equal to 14 c.c. of a 1.5 per cent. solution. Four per cent. of 70 kg. is equal to 2.8 kg., and without making a correction for the difference in specific between the blood plasma and water, this would amount to, roughly, 2,800 c.c. If 210 mg. are dissolved in 2,800 c.c., then 7.5 mg. should be dissolved in 100 c.c.

with 0.8 per cent. saline. One part of this is mixed with one part of the patient's plasma and two parts of salt solution, yielding a dilution of 1 in 4. With this solution, the prism of the colorimeter (Rowntree and Geraghty's modification of the Autenrieth-Königsberger apparatus) is filled. This dilution has been found to be well adapted to colorimetric determinations. Each of the specimens (2 and 3 of the patient's plasma) is diluted with three parts saline and placed in the cup of the colorimeter and read against the above standard.

CALCULATION OF RESULTS

The method described above furnishes volume values for plasma and total blood. When it is desired to express values as per cent. of body weight, the formulas given below can be utilized. Since the specific gravities of the plasma and of the blood were not ascertained in this study, one of 1.050 for the whole blood and of 1.030 for the plasma have been used.

FORMULAS

Plasma Volume.—Weight of patient in kg. = W.

Standard prepared so that amount of dye injected is diluted in 40 c.c. for every kg. of body weight.

Reading of standard prepared as directed = 100.

Reading of patient's plasma against standard = R.

The number of c.c. of plasma in patient's body = $100/R \times 40\ W = 4{,}000\ W/R$.

Percentage of Body Weight.—Plasma volume in c.c. × Sp. G. of plasma/1,000 = weight of plasma = X.

$X \times 100/W$ = per cent. of body weight.

Cubic centimeters of plasma per kg. = No. c.c. in body/W.

Total Blood Volume.—Let hematocrit reading of the volume occupied by corpuscles = P.

Volume of plasma = 100 — P.

Total blood volume = plasma volume × 100/100 — P.

Total Blood as Per Cent. of Body Weight.—Total blood volume in c.c. × Sp. G./1,000 = weight of total blood = Y.

$Y \times 100/W$ = per cent. of body weight.

Cubic centimeters per kg. = number c.c. in body/W.

UNDESIRABLE FEATURES OF THE METHOD

Hemolysis.—Sometimes the colors do not match perfectly in quality. This is the result of hemolysis and of necessity makes the quantitative determination more difficult. It is extremely infrequent, however, to find this failure with both specimens, and on the one or two occasions when this has occurred, the determination was not attempted. Records have been kept throughout as to the matching of the colors. In a large proportion of instances the colors in both specimens were perfect. Where the matching was only slightly imperfect, readings were made and in many instances these have closely approximated readings of the other specimen in which the color was perfect. In

spite of the most careful technic, inexplicable hemolysis occurred at times. It is considerably more frequent in the narrow graduated centrifuge tube used for the hematocrit values.

Lipemia.—Lipemia, if very marked, makes the determination impossible. Only once has this been encountered. In a few instances it offered some slight difficulty. By proper attention to diet and to the time of making the determination, this difficulty can be readily obviated. In our series it has not been sufficiently troublesome to require such measures.

Discomfort to Patient.—Aside from the slight pain of the venepuncture, the patient usually suffers no discomfort or inconvenience. In approximately 9 per cent. of the cases, chilly sensations occurred, while in five instances, chills, with fever reaching to 101, were seen. None of the normal cases exhibited thermic responses. They are apparently more common in cases of anemia.

CONTROLS OF THE METHOD

Controls on the method have been made in order to determine the constancy of its findings under various conditions and in order to justify certain steps in the technic. These studies are here presented under subheadings. Controls on the plasma method are first considered and later those on the hematocrit method of determining total blood volume.

A. Rate of Disappearance of Dye from Blood.—Throughout the work, readings have been made at three minute and six minute intervals. The following afford striking proof of the slowness with which the dye disappears from the circulation. In a series of thirty-six cases in which the color matching was equally good in both determinations, nine cases showed identical reading for the two determinations, nine showed higher readings for the second, the average amount above the first being 2.5 per cent., while eighteen showed higher readings for the first determination, the average amount above the second being 3 per cent. This indicates that some dye does disappear from the circulation during the time of determination, but also indicates that the loss is inconstant and small. On rare occasions, marked discrepancies in the two readings occur. Such determinations should be discarded.

Below is shown the rate of disappearance of the dye from plasma in a man over a period of twelve minutes, readings being made at approximately two-minute intervals, and the amount of dye of the first determination being considered 100 per cent.

J. C., weight 55.4 kg.; 178 mg. dye injected.

Time Elapsing Minutes[5]	Per Cent.
2	100
4	104
6	103
8	102
10	102
12	97

Rate of Disappearance of Dye from the Circulation in Dogs.—Protocols 1 and 2 indicate the slow rate of disappearance of dye from the blood of dogs, the amount present on first determination being considered as 100 per cent.

Dog 1.—Weight, 7.7 kg.; 23 mg. dye injected.

Time Elapsing Hours, Minutes	Per Cent.
.. 13	94
.. 30	92
2 20	65
3 ..	60
6 ..	52
24 ..	26

Dog 2.—Weight, 9.5 kg.; 100 mg. dye injected.

Time Elapsing Hours, Minutes	Per Cent
.. 2	93
.. 4	93
.. 10	86
.. 12	86
.. 15	81
1 6	77
1 8	73

These data demonstrate that the absorption from the blood stream is slow, and justify us in accepting determinations made within six minutes of the time of injection of the dye. Further proof of the slowness of the absorption is shown by the fact that on several occasions the plasma three to four days after injection showed a considerable amount of the dye. The urine also shows traces for some days after the injection.

B. Values Found on Repeated Determinations.—Repeated determinations on the same individual yield practically identical results, provided no appreciable change in the patient's condition has occurred. Some of these persons are normal and others are suffering from chronic disease in which no appreciable change occurred during the interim.

5. The first reading at two minutes was lower than subsequent ones. Mixing was probably not complete in two minutes.

TABLE 1.—Repeated Determinations on Men

Name	Date	Weight	Volume in c.c.	Cubic-centimeters per Kg.
Cl.	2/ 2/15	58	2,922	50
	2/ 4/15	58	3,030	52
Fl.	2/ 2/15	67	2,969	44
	2/ 4/15	67	3,037	45
D.	3/11/15	57	2,665	47
	3/12/15	57	2,681	47
G.	3/11/15	64	3,176	50
	3/12/15	64	3,083	48
B.	1/ 7/15	61	3,036	49
	2/ 6/15	64	3,165	49
T.	2/12/15	50	1,975	40
	2/25/15	50	1,990	40
Fr.	1/20/15	62	3,056	49
	3/18/15	63	3,160	50
I.	1/12/15	81	3,172	39
	2/ 3/15	80	3,064	38
W.	4/22/15	64	3,375	53
	5/ 3/15	65	3,400	53

Dogs do not give so constant results but it is altogether probable that the dogs' plasma volume is subject to relatively greater and more sudden changes than that of man. Some interesting findings on dogs are included in Table 2.

TABLE 2.—Repeated Determinations on Dogs

No.	Date	Weight	Plasma, c.c.	C.c. per Kg.	Remarks
1	1/19	10.5	696	66.3	
	1/27	9.35	623	66.6	Distemper
	2/ 1	8.1	572	70.6	
	2/ 3	7.65	481	62.8	Following immediately a bleeding of 165 c.c.
	2/ 4	7.62	651	85.5	Next day
			958	126	After infusion of 325 c.c.
2	1/27	9.05	603	66	
	2/15	9.02	635	61	
4	1/19	15	1,202	81.1	Distemper — bled 300 c.c. six minutes before first sample
	1/21	15	1,137	76.4	

C. Plasma Volume Determined with Varying Amounts of Dye.—The same value for blood volume is obtained whether one works with 2, 3 or 4 mg. per kilogram. Three practically normal men were studied

in this connection. Determinations made after injection of 2 and 3 mg. doses yielded values within 75 c.c. in two instances, while in the third, 2 and 4 mg. per kilogram were used, the results agreeing within 1 per cent. Three mg. per kg. is considered absolutely satisfactory, yielding colors well adapted to colorimetric determination.

D. Controls on the Colorimeter.—In the past, in making parallel series of readings with various phthaleins and with Folin's micro-Kjeldahl method, practically identical readings were obtained with two instruments, the Duboscq and our colorimeter. In a small series of parallel readings made in this investigation, identical readings were also obtained with both.

E. The Loss of Blood at Venesection Demonstrated.—The method was applied to three men before and after phlebotomy. The results appear in Table 3. Nos. 1 and 2 were normal donors for transfusion and No. 3 was a hypertensive patient, the subject of a therapeutic venesection.[6]

TABLE 3.—Loss of Blood at Venesection

Name	Volume Before	Amt. Bled	Volume After
1. G.	Plasma, 3,180	300	Plasma, 2,862
2. D.	Plasma, 2,790	620	Plasma, 2,573
	Blood, 5,083		Blood, 4,274
3. A.	Plasma, 3,872	800	Plasma, 3,425
	Blood, 6,143		Blood, 5,434

A decrease corresponding fairly well to the amount of blood removed is demonstrated in all three cases.

F. Hematocrit.—The whole blood volume is calculated from the plasma volume on the basis of the hematocrit findings, after centrifugalization in graduated tubes for 3,000 revolutions per minute for twenty minutes. A longer period was unnecessary, the packing always reaching its maximum within this time. The values from the same individual varied but little, whether the samples were obtained with free circulation of blood or after impeding the venous return for as long as fifteen minutes. When the venous return was cut off, the red cells increased 4 per cent. and 5 per cent. in two instances and decreased 2 per cent. and 5 per cent. in two others. Where possible, it is better to take sample for hematocrit determination from the freely

6. The Lindeman method of transfusion was used and the amount of blood measured.

circulating blood. Repeated determinations on the same normal individual give close but not identictal values. Undoubtedly some opportunity for error is here afforded.

G. Is the Dye Taken Up by the Red Blood Cells?—Early in the course of this work it became evident that the dye was not readily taken up by tissue cells or the cells in the blood stream. From the experiments with collodion sacs previously mentioned and from the fact that neither the erythrocytes nor leukocytes were stained when observed in fresh blood preparations, it seemed fair to surmise that most of the dye remained in the plasma during the short time of the determination. In order to get at this question quantitatively, it was first necessary to determine the relative amounts of plasma and red blood corpuscles in the whole blood. Several methods have been employed for this purpose, among others, the various forms of the hematocrit and the nitrogen method devised by Bleibtreu.[7] The hematocrit method described above was found to be the most satisfactory for our purpose, yielding figures that agreed closely with those obtained by other workers with this method. In two different samples of blood, the relative amounts of plasma and erythrocytes were determined by the hematocrit and by the nitrogen method. The latter gave a plasma value 4 per cent. to 9 per cent. higher than that of the hematocrit. Bleibtreu's figures were also high compared with those of other observers using the hematocrit method. In our work, the hematocrit values only were taken.

In order to determine whether the dye is absorbed by the blood cells, and, if so, to what extent, the following procedure was adopted: Fifty c.c. of blood were taken from a healthy man and rendered incoagulable with powdered sodium oxalate. Ten c.c. of this blood were used for hematocrit determination, 15 c.c. being centrifuged and the plasma pipetted off. The remainder was agitated until measured out for the experiments given below:

Experiment 1.—Centrifuge Tube 1. Fifteen c.c. of this whole blood pus 2 c.c. of dye solution plus 13 c.c. of saline.

Centrifuge Tube 2.—Five c.c. of plasma plus 1 c.c. of dye solution plus 4 c.c. of saline.

After Tube 1 was centrifugalized,[8] the plasma was pipetted off and compared with the plasma dye mixture from Tube 2, which was placed as the standard in prism of the colorimeter. The reading was 80. Therefore, in dilutions used, Tube 1 contained 80 per cent. as much dye per cubic centimeter as Tube 2. The relative amount of plasma in the blood in Tube 1, as shown by the hematocrit, is 62.5 per cent.

Then $15 \times 62.5 = 9.37$ c.c. plasma in Tube 1.

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7. Bleibtreu, M. and L.: Eine Methode zur Bestimmung des Volums der körperlichen Elemente im Blut, Arch. f. Physiol. (Pfluger's), 1892, li, 151.

8. Three thousand revolutions per minute for twenty minutes.

Then 2 c.c. of dye solution is diluted in 24.37 c.c., or 1 c.c. in 12.18 c.c.
Or there should be 10/12.18, 82.9 per cent. of dye in Tube 1.
Colorimeter reading, as calculated from hematocrit data, 82.9 per cent.
Colorimeter reading observed, 80 per cent.

Experiment 2.—Experiment 1 was repeated with the blood taken from another normal individual.

Colorimeter reading, as calculated from hematocrit data, 81.8 per cent.
Colorimeter reading observed, 82 per cent.

Experiment 3.—Experiment 1 was repeated with the blood taken from a third normal individual.

Colorimeter reading, as calculated from hematocrit data, 81.4 per cent.
Colorimeter reading observed, 81 per cent.

In considering the foregoing experimental data, several factors should be noted. The hematocrit must necessarily give us the minimum value for the amount of plasma in the blood, as the corpuscles are wet and the interstices are filled with fluid. Therefore, the hematocrit findings for plasma must be slightly low, and the calculated colorimeter reading slightly high. If the dye is absorbed by or diffuses into the corpuscles, the observed colorimeter readings will be low, that is, the plasma volume large. As the calculated and observed figures agree so closely, the amount of dye lost from the plasma must be negligible. From the foregoing experiments, it would seem safe to conclude that in vitro little or none of the dye passes into the blood cells.

RELATION OF BLOOD AND PLASMA VOLUME TO BLOOD PRESSURE

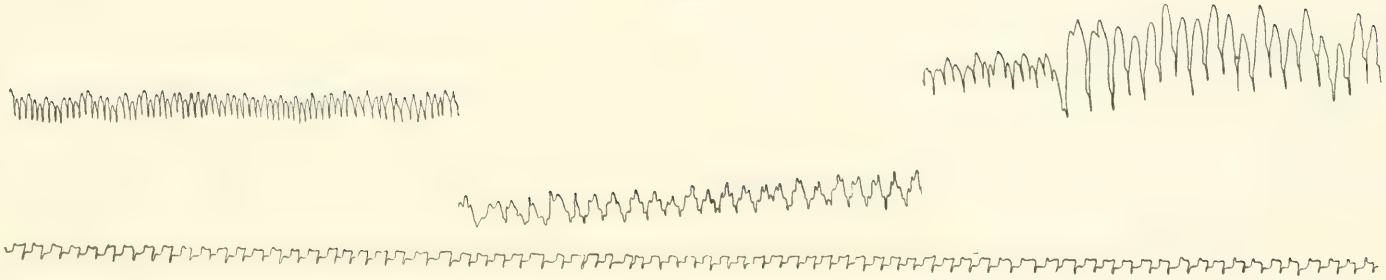
At the suggestion of Dr. Janeway, experiments were carried out to ascertain what effects changes in the caliber of the smaller blood vessels have on blood and plasma volumes. Volume determinations were made on dogs attached to a kymograph. The tracings with the blood values appear in Figures 1 and 2. The vasoconstriction was obtained by continuous administration of epinephrin, a fairly constant level being maintained for five minutes. In each determination, before injecting the dye, plasma was removed for the standard and within five minutes after the injection the samples were withdrawn for the volume estimations. The dogs weighed 14.3 and 12.7 kg. respectively.

No striking change in blood or plasma volume accompanied the fairly marked variations in blood pressure. However, these blood pressure changes were of short duration.

Dr. P. D. Lamson,[9] working in Dr. Abel's laboratory, has found that the polycythemia which develops after epinephrin injection does not reach its maximum for from fifteen to twenty minutes. Volume determinations made on two dogs after this interval showed a slight but definite decrease in plasma volume.

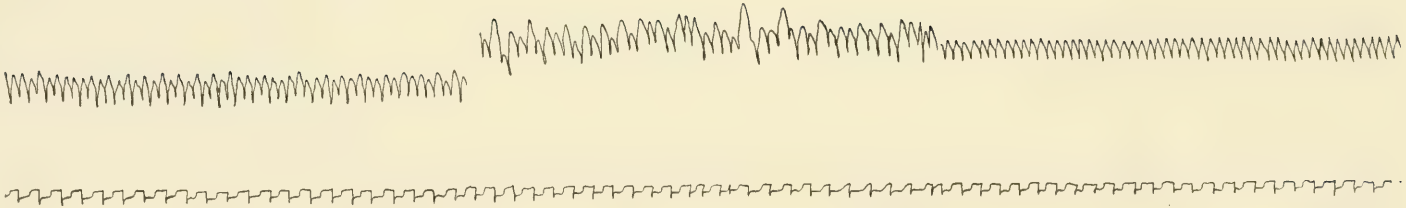
9. Personal communication.

Figure 1



Plasma	533 c.c.	591 c.c.	524 c.c.
Blood	1,278 c.c.	1,334 c.c.	1,175 c.c.
Blood pressure	126 mm.	60 mm.	170 mm

Figure 2



Plasma	605 c.c.	610 c.c.	624 c.c.
Blood	1,440 c.c.	1,420 c.c.	1,450 c.c.
Blood pressure	120 mm.	165 mm.	160 mm.

METHODS PREVIOUSLY EMPLOYED AND VALUES OBTAINED

According to Welcker,[10] the earliest attempt to determine the blood volume was made by Haller.[11] It consisted of bleeding two criminals to death, the amounts of blood obtained being 28 and 39 pounds.

The methods fall into two general groups, only those of Group 2 being applicable for clinical purposes.

1. *Direct Method.*—Welcker, in 1854, bled animals to death, washed out the blood vessels with water, minced and washed the tissues thoroughly, brought the blood and washings together and determined their hemoglobin content. He concluded that the blood constituted one-thirteenth of the body weight in mammals. Shortly after this, Bischoff[12] applied this method to two criminals and obtained the same value. Modifications of the method were made by Heidenhain[13] and by Plesch.[14] A similar preliminary procedure was used by Cohnstein and Zuntz,[15] the calculations being based on cell counts instead of hemoglobin estimations, and by Kottmann,[16] who employed the hematocrit.

2. *Indirect Methods.*—A. Infusion method: (*a*) Herbst,[17] in 1822, injected the vascular system of a corpse until the vessels seemed moderately filled. (*b*) Vierordt[18] experimented on animals. He determined the amount of blood pumped from the heart in one second and multiplied this by a number corresponding to the seconds required for a complete circulation. On the basis of certain calculations, he concluded that in man the blood constituted one-thirteenth of the body

10. Welcker, H.: Bestimmungen der Menge des Körperblutes und der Blutfärbekraft, sowie Bestimmungen von Zahl, Maass, Oberfläche und Volumen des einzelnen Blutkörperchens beim Thier und beim Menchen, Präger Vrtljschr., 1854, iv, 11; Ztschr. f. rat. Med., 1858, iv, 145.

11. Quoted from Welcker (Note 10).

12. Bischoff, L. W.: Bestimmung der Blutmenge bei einem Hingerichten, Ztschr. f. Wissensch. Zool., 1856, vii, 331; Abermalige Bestimmung der Blutmenge bei einem Hingerichteten, ibid., 1858, ix, 65.

13. Heidenhain: Ueber die Blutmenge der Säugethiere mit besonderer Rücksicht auf Welcker's Methode der Blutbestimmung, Arch. f. physiol. Heilk., 1857, n. f., i, 507.

14. Plesch, J.: Hämodymamische Studien, Ztschr. f. exper. Path. u. Therap., 1909, vi, 380.

15. Cohnstein and Zuntz, N.: Untersuchungen über den Flüssigkeits-Austausch zwischen Blut und Geweben unter verschiedenen physiologischen und pathologischen Bedingungen, Arch. f. Physiol. (Pfluger's), 1888, xlii, 303.

16. Kottmann, K.: Ueber die Bestimmung der Blutmenge beim Menschen und Tier unter Anwendung eines neuen Prazisionshaematokriten, Arch. f. exper. Path. u. Pharmakol., 1906, liv, 356.

17. Quoted from Welcker.

18. Vierordt, K.: Das Abhängigkeitsgesetz der mittleren Kreislaufszeiten von den miltleren Puls-frequenzen der Thierarten, Arch. f. physiol. Heilkunde, 1858, n. f. ii, 527.

weight. (*c*) Valentin[19] removed a definite amount of blood (a), then injected a known amount of water into the vascular system and bled again (b). The solids of a and b were determined, the difference being due to the number of cubic centimeters of water injected. Weber and Lehmann,[20] utilizing this method, concluded that the blood constituted one-eighth of the body weight. (*d*) Quincke[21] utilized the method of Mallassez.[22] Red counts were made in cases of pernicious anemia. A known amount of blood with a known cell count was transfused, following which the patient's red cells were again counted. From these data, the volume was calculated.

B. Inhalation Method: Gréhant and Quinquaud[23] gave dogs by inhalation a measured volume of carbon monoxid and determined its percentage content in a small sample of the animal's blood. Haldane and Smith[24] modified this method and applied it clinically. The patient breathes for five minutes a measured quantity of carbon monoxid from a specially devised apparatus. A sample of blood is removed and its carbon monoxid content estimated by the carmin titration method of Haldane. Plesch[25] and Oerum[26] have employed this method, but the former determined the carbon monoxid volumetrically.

C. Antitoxin Method: Behring[27] noted that tetanus antitoxin remained in the circulation for long periods and made this the basis for the estimation of blood volume. His studies showed the blood to constitute 1/11.8 of the body weight. With it, Kämmerer and Waldmann[28] in Müller's clinic, obtained a value of 1/10.2, while Fries[29] found a value of 1/12.6.

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19. Valentin, G.: Lehrbuch der Physiologie, 1847, i, 493.

20. Weber, E., and Lehmann, C. G.: Lehrbuch der physiologische Chemie, 1850, ii, Auflage ii, 259.

21. Quincke: Weitere Beobachtungen über perniziose Anamie, Deutsch. Arch. f. klin. Med., 1877, xx, 27; ref. Plesch.

22. Malassez, L.: Nouveaux procédés pour apprécier la masse totale du sang, Arch. de physiol. norm. et path., 1874, vi, Série 2, 797.

23. Gréhant and Quinquaud: Mesure du volume de sang contenu dans l'organisme d'un Mammifère vivant, Compt. Rend. Acad. Sc., 1882, xciv, 1450.

24. Haldane, J., and Smith, J. Lorrain: The Mass and Oxygen Capacity of the Blood in Man, Jour. Physiol., 1899-1900, xxv, 331. Smith, J. Lorrain: Discussion on the Blood in Disease, Trans. Path. Soc., London, 1900, li, 311.

25. Tarchanoff, J. R.: Die Bestimmung der Blutmenge in lebenden Menschen, Arch. f. Physiol. (Pfluger's), 1880, xxiii, 548; 1881, xxiv, 525.

26. Oerum, H. P. T.: Quantitative Blutuntersuchungen, Deutsch. Arch. f. klin. Med., 1908, xciii, 356.

27. v. Behring, E.: Die Antitoxinmethode zur Blutmenge Bestimmung, München. med. Wchnschr., 1911, lviii, 655.

28. Kämmerer, H., and Waldmann, A.: Blutmengebestimmungen nach v. Behring und andere quantitative untersuchungen der Blutbestandteile, Deutsch. Arch. f. klin. Med., 1913, cix, 524.

29. Fries, H.: Ueber Veränderungen der Blutmenge in Schwangerschaft, Geburt und Wochenbett, Ztschr. f. Geb. u. Gyn., 1911, lxix, 340.

Table 4 indicates the wide variations that have been obtained by the different methods. The total number of normal cases has been small. In the report of Haldane and Smith,[24] the limits for normal are very wide. They state specifically, however, that one of the cases was very obese, that is, the one in which the blood was only one-thirtieth of his body weight.

TABLE 4.—Variations in Blood Volume by Different Methods

Author	Method	No. of Cases	Extreme Fractions	Average	Per Cent Body Weight	Remarks
Haller.....	Bled to death; weight of blood	2	.....	.....	.....	24-30 pounds of blood. Body wt. not given
Welcker and Bischoff	Bled and washed out vessels of criminals. Compared with hematin	3	1/14.6-1/12.4	1/13	7.6	This was accepted standard from 1858 to 1881
Tarchanow*	Sweating and counting R. B. C. and Hb. before and after steam bath	7	1/11.7-1/21.8	1/15	6.6	
Vierordt....	No. c.c. put out by heart multiplied by No. of seconds required for complete circulation	.....	.....	1/13	.....	Worked on horses and dogs. Error, 12½ per cent.
Weber and Lehmann	Infused water, washed out vessels; determined dry substance of blood	2	.....	1/8	12.5	
Haldane and Smith	CO inhaled. Carmin titration	12	1/30-1/16	1/21	3.34-6.27	
Kottmann †	Infusion of salt sol. and hematocrit before and after	4	1/11.5-1/13	1/12.2	8.2	
Smith‡.....	CO method. Titration with carmin solution	6 women 12 men	....	....	4.4-6.1¶ 3.3-6.27	
Oerum.....	CO method. Titration with carmin solution	9 men 4 women	....	....	3.7-8.3¶ 2.5-6.6¶	
Plesch.....	1. Infusion with Hb. determinations. 2. CO inhalation with combustion	21 4	1/21-1/16.5 1/20-1/16.5	1/19 1/18	4.69-6.05 5.02-6.05	15 % error
Kämmerer and Waldmann	Behring method.....	7	1/11.1-1/9.3	1/10.2	9-10.7	
Fries.....	Behring method.....	10	1/17.5-1/11	1/12.6	7.9	Other practically normal patients gave similar values

* Arch. f. physiol., 1880, xxii, 548; 1881, xxiv, 525.
† Arch. f. exper. Path. u. Pharmakol., 1906, liv, 356.
‡ Tr. Path. Soc. London, 1900, li, 311.
¶ c.c. per 100 grams of body weight.

NORMAL AND PATHOLOGICAL VARIATIONS IN BLOOD AND PLASMA VOLUME IN MAN

Results (Normal)

At least 140 determinations for plasma volume have been made on more than 100 individuals. In the earlier work, hematocrit studies were not made, so that the values for total blood volume are considerably fewer.

Normal Values.—The results here reported are for normal men, being obtained from patients attending the outpatient department of the genito-urinary clinic for some minor local condition, such as chronic urethritis, chronic prostatitis or verumontanitis, etc. The age, weight, height and blood pressure, as well as the blood values, will be seen in the accompanying tables (5 and 6).

TABLE 5.—Plasma Values in Twenty-Four Subjects

No.	Name	Date	Age	Weight, Kg.	Height, Ft.	In.	B. P. Systolic	Volume, c.c.	C.c. per Kg.	Fraction Body Weight	Per Cent Body Weight
1	F. J. ...	1/2	39	58.8	5	3	125	3,100	45	1/21.6	4.6
2	E. M. ..	1/6	22	69.5	5	10	...	2,775	47	1/20.7	4.8
3	G. P. ..	1/7	40	56.1	5	6	115	3,050	53	1/18.3	5.4
4	M.	1/7	39	51.8	5	11	...	2,700	52	1/18.6	5.3
5	P.	1/9	34	73.5	5	3	138	3,050	42	1/23	4.3
6	V.	1/9	23	58.6	5	7	90	3,050	52	1/18.6	5.3
7	C.	2/2	..	53.4	.....		...	2,925	55	1/17.6	5.6
		2/4	..	53	.....		...	3,025	57	1/17	5.8
8	F.	2/2	..	67	.....		...	2,975	43	1/22	4.5
		2/4	..	67	.....		...	3,050	45	1/21.6	4.6
9	S.	1/29	25	68.4	5	6	...	3,100	45	1/21.6	4.6
10	H. S. ..	12/28	33	58.9	5	5	90	2,975	50	1/20	5
11	P. B. ..	12/28	51	72	5	6	122	3,275	45	1/21.6	4.6
12	J. J. ...	12/28	22	57.5	5		...	2,800	49	1/19.8	5
13	J. S. ...	12/28	46	63.6	5	3	142	3,075	48	1/20.4	4.9
14	J. C. ...	12/29	43	71	5	6	130	3,325	48	1/20.4	4.9
15	H. O. ..	12/29	30	71	5	10	...	3,150	45	1/21.6	4.6
16	B. S. ...	12/31	32	65.3	5	6	145	3,100	47	1/20.7	4.8
17	G. D. ..	12/31	36	62.5	5	10	120	3,100	50	1/20	5
18	G.	1/2	34	69.1	5	8	130	3,300	48	1/20.4	4.9
19	F. L. ..	1/2	32	50.9	5	5	105	2,500	49	1/19.8	5
20	F. S. ...	12/30	49	66.8	6		130	3,200	48	1/20.4	4.9
21	E. J. ...	1/5	..	70.5	6	2	...	3,750	53	1/18.3	5.4
22	J. M. S.	3/11	..	52.3	.....		...	2,675	51	1/19	5.2
		3/12	..	52.3	.....		...	2,675	51	1/19	5.2
23	D.	1/22	..	70.9	5	11	130	3,375	48	1/20.4	4.9
24	S.	1/12	..	56.4	5	7	...	2,825	50	1/20	5

TABLE 6.—Blood Values in Eighteen Normal Subjects

No.	Name	Age	Date	Weight, Kg.	Plasma Volume, c.c.	C.c. per Kg. Body Weight	Fraction Body Weight	Per Cent. Body Weight	Hematocrit		Total Blood Volume	C.c. per Kg. Body Weight	Fraction Body Weight	Per Cent. Body Weight
									R. B. C.	Plasma				
25	H. G.	..	3/11/15	64.3	3,175	49.4	1/20	5	38.3	61.7	5,125	79	1/12	8.3
			3/12/15	64.3	3,075	48	1/20.4	4.9						
26	J. T.	36	4/24/15	85	3,775	44	1/22	4.5	43.4	56.6	6,675	78.5	1/12	8.2
27	W.	24	4/24/15	76.3	3,525	46	1/21	4.7	44	56	6,300	82	1/11.6	8.6
28	H. S.	..	5/ 7/15	61.8	3,225	52	1/19	5.3	45.4	54.6	5,900	95	1/10.5	9.9
29	W.	..	4/21/15	59.5	2,975	50	1/20	5.1	37.6	62.4	4,775	80	1/12	8.4
30	W t.	53	5/ 3/15	65.3	3,400	52	1/18.3	5.3	41.6	58.4	5,775	88	1/11	9.2
			4/22/15	63.5	3,375	53	1/18.3	5.4	40	60	5,625	88	1/11	9.2
31	B.	26	4/28/15	63.6	3,025	48	1/20.4	4.9	48	52	5,825	92	1/10.4	9.6
32	J.	24	4/28/15	56.8	2,850	50	1/20	5.0	45.7	54.3	5,250	92	1/10.4	
33	G.	..	4/28/15	60.9	3,300	54	1/17.9	5.5	40.5	59.5	5,500	91	1/10.4	9.5
34	L.	28	4/28/15	60.7	3,200	52	1/18.3	5.3	46.9	53.1	6,025	99	1/19.6	10.4
35	E.	20	4/28/15	72.5	3,575	49	1/20	5.0	38.6	61.4	5,825	80	1/12	8.4
36	L.	21	5/ 2/15	66.5	2,874	43	1/22.7	4.4	49	51	5,725	84	1/11.4	8.8
37	H. B.	23	5/ 3/15	47.4	2,850	52	1/18.1	5.3	43	57	4,200	88	1/11	9.2
38	R. K.	25	5/ 3/15	72.3	3,300	46	1/21.3	4.7	45	55	6,000	83	1/11.5	
39	J. C.	33	5/15/15	55.5	2,775	50	1/20	5	45	55	5,025	90	1/10.5	9.4
			5/ 7/15	56.8	2,850	50	1/20	5	40.4	59.6	4,775	84	1/11.4	8.8
40	G. P.	40	1/ 7/15	56.1	3,040	53	1/18.5	5.4	32.7	67.3	4,525	80	1/11.9	8.4
41	W. M.	39	1/ 7/15	51.8	2,700	52	1/18.7	5.3	38.2	61.8	4,375	84	1/11.3	8.8
42	E. M.	22	1/ 6/15	69.5	3,275	47	1/20.8	4.8	42.9	57.1	5,725	82	1/11.5	8.7

Plasma Values.—Forty-eight determinations were made on forty-two patients. The largest (3,775 c.c.) and smallest (2,450 c.c.) volumes were encountered in the heaviest (85 kg.) and lightest (47.4 kg.) patients (Nos. 26 and 36 respectively). The mean weight was 61.2 kg. and the mean volume 3,125 c.c.; the average weight, 63.3 kg. and the average volume 3,050 c.c. The plasma varied from 42 to 56 c.c. per kilogram, while the fraction of body weight varied from 1/23 to 1/17, the average being 1/19.4. (From this it appears that a normal man of 140 pounds has 3 liters of plasma, or 50 c.c. per kg.) The constancy in the relation of plasma to body weight in health is striking, there being no great extremes.

Blood Values.—The number of determinations of total blood values has not been so large. Twenty-one determinations on eighteen normal individuals were made, the whole blood values being calculated from the plasma volume and hematocrit findings. The plasma constitutes on the average 56.9 per cent. of the total blood, the extremes being 51 per cent. and 62.6 per cent. The largest and smallest volume (6,675 c.c. and 4,200 c.c.) were obtained in the two patients mentioned above as having the largest and smallest plasma volumes. These patients showed 78.5 and 97 c.c. per kilogram, respectively. The average blood volume was 5,350 c.c., or approximately 85 c.c. per kilogram. On a gravimetric basis, the blood constituted 8.8 per cent., or 1/11.4 of the body weight. The variations in health were not great—from 1/13 to 1/10.5 of the body weight.

The constancy of these values is in striking contrast to those of Haldane and Smith[24] and of Oerum,[26] who found the blood-body-mass relationship exceedingly variable. The results here reported support the figures of Welcker and Bischoff, Vierordt, Kottmann, Behring, Fries, and Kämmerer and Waldmann.

Clinical Studies (Pathological)

Sixty-five patients were studied, the determinations numbering eighty-eight.

For purposes of discussion the cases are grouped according to clinical conditions. It is to be regretted that the number of cases in each group is not larger. This was impossible, since the drug is of German origin and despite repeated efforts it was impossible to secure a further supply.[30] The scope of the work has been limited by the small amount of dye available.

30. It is probable that many dyes will answer the purpose equally well. Studies along these lines are now in progress.

TABLE 7.—Blood Observations Before and After Delivery

No.	Name	Age	Para	Relation of Observation to Labor, Days		Blood Lost	Weight of Child	Body Weight	Plasma Volume	C.c. per Kilo	Blood Volume	Per Cent. of Body Weight	Per Cent. of Estimated Weight	Estimated Decrease in Blood Volume	Hematocrit Volume R.B.C.
				Before	After										
1	L. V.	21	0	2	...	210	4,165	55.5	3,213	57.9	5,637	10.66	11.16	.....	43
					5	...	.....	48.2	2,487	51.6	4,363	9.50	.....	1,275	43
2	G. Z.	30	1	19	...	100	4,150	61	3,343	54.8	5,306	9.13	10	.....	37
					4	...	.....	52.7	3,057	58	4,700	9.36	.....	506	35
					11	...	.....	53.9	2,641	49	4,192	8.1	.....	1,286	37
3	M. B.	16	0	13	...	150	3,315	51.8	2,621	50.6	3,971	8.05	8.5	.....	34
					4	...	.....	45.4	2,243	49.4	3,299	7.6	....	672	32
4	E. J.	20	0	9	...	325	3,100	57	3,329	58.4	5,044	9.03	9.6	.....	34
					1½	...	.....	50	3,030	60.6	4,734	9.94	....	310	36
					9	...	.....	50.5	2,525	50	3,945	8.2	....	1,099	36
5	C. O.	23	3	6	...	250	3,970	65.6	3,870	59	5,691	9.11	9.7	.....	32
					4	...	.....	55	2,695	49	4,022	7.68	....	1,569	33
6	F. S.	25	2	4	...	800	3,085	63.9	3,623	56.7	6,039	9.92	9.5	.....	40
					3	...	.....	57.3	2,974	51.9	4,721	8.65	....	1,318	37
7	E. T.	22	1	39	...	200	4,075	64.5	4,689	72.7	7,105	11.55	12.5		
					9	...	.....	60	3,078	51.3	4,809	8.41	....	2,296	36
8	L. L.	22	2	4	...	100	3,640	61.4	3,899	63.5	5,262	8.98	10.2	.....	28
					7	...	.....	59.3	2,894	48.8	4,904	8.68	....	358	41
9	B. W.	18	0	11	...	225	3,420	61.7	2,974	48.2	4,647	7.89	9.4	.....	36
					5	...	.....	59.1	2,216	37.5	3,821	6.79	....	826	42
10	A. McD.	20	0	1	...	675	3,420	63	3,717	59	6,300	10.5	11.4	.....	41
					8	...	.....	57	2,519	44.2	3,760	6.9	....	2,640	43
11	M. F.	16	0	6	...	200	1,040	47.1	1,795	38.1	3,042	6.78	....	.....	41
					10	...	.....	43.6	1,548	35.5	2,669	6.43	....	373	42
12	E. Z.	17	0	61	...	...	.....	54.5	3,461	63.5	5,408	10.42	....	.....	36
13	M.	19	0	147	...	...	.....	57.3	3,266	57	5,184	9.51	....	.....	37

Pregnancy.—Observations have been made before and after delivery in eleven cases (Table 7). In the latter months of pregnancy, the blood mass in proportion to the body weight is definitely increased. Whereas, normally, blood constitutes 8.8 per cent., in twelve pregnant women prior to labor the blood averaged 9.56 per cent., which corresponds to the highest limits of normal value. In four instances, the blood values rose considerably above normal limits. The plasma volumes were correspondingly increased, averaging 58.4 c.c. per kilogram, instead of 50 c.c. Determinations made a week to ten days after labor showed that a great decrease in the blood mass had occurred which could not be accounted for by loss of weight (child, placenta and amniotic fluid) or loss of blood at parturition. After labor, the blood and plasma formed 9 per cent. and 4.9 per cent. of the body weight, respectively. The average loss of blood, according to the obstetrical report of these cases, was only 300 c.c., whereas blood volume studies showed an average loss of 1,100 c.c.

Efforts have been made, but without success, to find the dye in the placenta, in the amniotic fluid and in the child's urine, following the administration of the dye intravenously to the mother during labor. Abnormal absorption of the dye would not seem to be the explanation of the high blood values recorded in pregnancy. This opinion is based on the fact that the decrease in blood mass is not found immediately after parturition, for in two cases (2 and 4) it will be seen that determinations made thirty-six hours in one case, and four days after delivery in the other, showed the same high values for plasma and blood. Subsequent determinations, ten days after labor, showed in both cases the marked decrease recorded in the other cases of the series. The consideration of blood volume in relation to pregnancy will form the subject of a separate communication in collaboration with Dr. J. R. Miller, with whom this part of the work was carried out in the wards of Prof. J. Whitridge Williams.

These findings confirm the widespread belief among obstetricians that pregnant women stand hemorrhage unusually well. Comparatively very large bleedings in eclampsia are well borne. It would appear that Nature has here a factor of safety, the large blood volume, safeguarding against the effects of hemorrhage before, during or after labor. Or it may be that the larger blood supply is necessary for proper nutrition of the child.

Case 11 was complicated by secondary lues and nephritis. It does not accord with the other cases of the series.

Emaciation.—Two cases (1 and 2, Table 8) are included: The first is in a woman who had lost 12 to 15 kg. through voluntary starvation. The plasma volume is absolutely normal, and since red cells were 4,000,000, her blood volume was, in all probability, not much below normal. The second patient, very tall and thin, also showed strictly normal values.

Obesity.—Six cases (Nos. 3, 4, 22, 26, 31 and 32, Table 8) showed in every instance a plasma volume decidedly below that of the average normal. The average was 37.1 c.c. per kg., or 3.8 per cent. of body weight, which is less than three-fourths of the normal value. In the one case (32) in which a hematocrit reading was obtained, 51 c.c. per kilogram of blood was found. This patient had only as much blood per kilogram as the normal individual has plasma per kilogram. Case 3 is of extreme interest, inasmuch as the plasma formed less than 3 per cent. of the body weight. These findings are in harmony with those of Haldane and Smith.

Emaciation and Anemia.—Ten cases (Nos. 5 to 14, inclusive, Table 8) exhibiting emaciation, in combination with moderate or severe secondary anemia, were studied. In three of these, malignant neoplasms were present. In six, the plasma volume was higher than the average normal, and in three, abnormally high. The average in eight cases was 56 c.c. per kilogram, which indicates a serous plethora. In four, the total blood volume was studied and three gave low values. It is evident, therefore, that in such conditions loss of plasma does not go hand in hand with loss of weight and decrease of red blood cells. Unfortunately, hematocrit studies were not made in the carcinoma cases. With the degree of anemia indicated in the table, it is evident, however, that the total blood volume must have been approximately normal.

Since the average blood value was 78 c.c. (1/13.2 of body weight), instead of 85 c.c. per kg., it becomes apparent that the anemia is fairly accurately indicated by the blood count. In Cases 8 and 11, there is a decrease in the volume, as well as in the cell content of the blood.

Pernicious Anemia.—Only three cases (Nos. 15, 16 and 17, Table 7) were studied. No. 15 showed an extremely high plasma volume of 72 c.c. per kg., the highest encountered outside of pregnancy. The blood volume was normal, but slightly below the average. In the other two, plasma values were normal, and the total volume considerably decreased.

With so great an increase in plasma volume in Case 15, it was thought that the whole blood mass might be larger than normal and be brought into causal relationship with the enlarged heart sometimes

seen in pernicious anemia. The two subsequent cases showed low blood values and no cardiac enlargement. Further data in this connection are necessary.

Polycythemia.—Only two cases (Nos. 18 and 19, Table 8) with high blood counts were studied. Patient 18, throughout several weeks' stay in the wards, presented a persistently high red cell count (8,000,000). Two determinations, a week intervening, yielded identical results, that is, both plasma and blood volumes were decidedly smaller than normal. This patient cannot, therefore, be considered plethoric, since he has only 73 c.c. of blood per kg., instead of the normal 85 c.c. per kg. The total number of cells and the absolute hemoglobin were probably about normal. The apparent plethora was caused by the low plasma content of the blood, 39 c.c. instead of 50 c.c. per kg.

Patient 19 showed a red blood cell count of 5,600,000, a count slightly above normal. This is an instance of true plethora. The plasma volume was also considerably above the average normal, being 60 instead of 50 c.c. per kilogram. The blood pressure was 135/90, while the blood was 113 c.c. per kilogram, instead of 85 c.c. per kilogram.

These cases strikingly demonstrate the necessity of total value for the proper interpretation of the routine methods of studying the blood.

Three other cases showed counts somewhat above the normal. In two of these, the plasma volume was low and the blood volume consequently not increased.

Diabetes.—Of the four patients whose cases were studied (Nos. 20 to 23, inclusive, Table 8), three were moderately thin men and one an obese woman. The three men showed a sugar content in the blood between 0.18 per cent. and 0.45 per cent., and all showed plasma and blood volumes that were strictly within the limits of normal. The obese patient (No. 22) showed blood volume decidedly below normal, so that the obesity exerted a greater influence on the blood-body-mass relationship than did the glycosuria. From these four cases it appears that an increase in the blood volume does not result from or accompany hyperglycemia.

Myocardial Insufficiency.—Nine plasma determinations were made on seven cases (Nos. 24 to 30, inclusive, Table 8) of myocardial insufficiency. Nephritis was absent, or played a very minor rôle in this series. Several of these patients had marked edema, and the average volume, 47 c.c. per kilogram, was slightly low, so that, in general, edema is not associated with an increase in plasma corresponding to the increase in weight. High values may occur, as is seen in Patient 30. The lowest plasma value (Case 27) is associated with a high red blood count.

TABLE 8.—CLINICAL (PATHOLOGICAL)—

No.	Name	Date	History No.	Age	Diagnosis	Red Blood Cells	Hgb. Sahli.	Weight	
								Actual	Best
1	A. E.	1/15	33581	32	Chronic pancreatitis. Malnutrition. Visceroptosis	4,022,000	82	36.8	48
2	E. J.	1/5	33514	24	Accident. Pain in side.....	5,100,000	100	70.5	...
3	R. M.	1/15	33583	48	Syphilis. Obesity	4,200,000	84	95.4	100
4	E. N.	1/6	33506	58	Obesity. Myocardial insufficiency, hypertension	5,000,000	80	107.7	159
5	R. B.	1/6	33467	66	Carcinoma stomach	2,600,000	45	38.8	...
6	J. H. C.	1/12	33559	72	Achylia gastrica; malnutrition. Arteriosclerosis	4,000,000	80	52.7	68
7	J. H.	1/6	33474	65	Carcinoma prostate. Metastases	2,120,000	45	48.6	...
8	A. E.	3/18	33818	31	Secondary anemia. Diarrhea. Psychosis. Migraine	3,600,000	61	43.9	52?
9	J. N.	1/15	33586	48	Carcinoma stomach	3,800,000	65	55.9	68
10	H. W.	4/9	.....	25	Balantidium coli. Secondary anemia	1,072,000	20	.....	...
11	J. S.	....	33040	22	Secondary anemia. Leg ulcers	1,500,000	31	50	...
12	R. A.	1/8	33522	54	Anacidity. Malnutrition. Secondary anemia	4,600,000	70	44.5	75
13	F. M.	3/12	33829	39	Duodenal ulcer. Secondary anemia	3,500,000	75	59.5	70
14	P. S.	2/22	33714	34	Acute nephritis. Syphilis. Secondary anemia	3,500,000	65	63	87
15	P. J. B.	1/5	33433	42	Pernicious anemia	1,192,000	28	68.2	...
16	T. A.	2/25	33770	38	Pernicious anemia	1,500,000	25	64.1	...
17	W. M.	1/23	C. H. I.	69	Penicious anemia. Aplastic type	1/17, 1,300,000; 1/28, 800,000	.. 22 15	53.6	...
18	G. T.	2/17	33642	22	Peritoneal adhesions. Polycythemia	8,000,000	100	50.5	55
		2/25	.....	..	.....	.....	..	.....	...
19	F. O.	1/5	33526	25	Mitral insufficiency. Left hemiplegia	5,600,000	101	69.1	72
20	G. V. H.	3/18	33876	23	Diabetes mellitus. Pulmonary tuberculosis. Coma	5,400,000	98	42.5	53
21	E. N. V.	3/11	33787	60	Diabetes mellitus. Pulmonary tuberculosis. Arteriosclerosis. Aortic insufficiency	4,700,000	82	58.2	76
22	M. B. J.	1/21	33487	37	Diabetes mellitus. Obesity.....	.....	85	80.9	80
23	L. W.	3/12	33817	29	Diabetes mellitus	5,500,000	89	54.1	67
24	B. S.	1/12	33571	44	Myocardial insufficiency. Pulmonary tuberculosis	5,200,000	100	78.2	84
25	O. O. M.	2/17	33735	60	Arteriosclerosis. Myocardial insufficiency	4,700,000	94	87.3	...
		2/25	.....	..	.....	.....	..	73.4	...
26	W. J. R.	1/15	33584	70	Myocardial insufficiency	5,000,000	84	89.5	...
27	J. S.	1/6	G. No. 98729	48	Syphilis. Aortic insufficiency. myocardial insufficiency	6,500,000	107	68.8	...
28	W.	1/23	.....	..	Myocardial insufficiency	.....	..	73.2	...
29	J. S. M.	1/9	33521	59	Arteriosclerosis. Angina pectoris. Myocardial insufficiency	5,000,000	80	89.5	112
30	J. F.	1/12	33531	42	Syphilis of aorta. Aortic insufficiency	.....	..	60.4	...
		1/22	.....	..	.....	.....	..	59.1	...
31	E. W. S.	2/4	33679	62	Pulmonary tuberculosis. Arteriosclerosis. Chronic nephritis	5,000,000	75	90.2	...

Edema	Blood Pressure	Cardiac Enlargement	Plasma c.c.	C.c. Per Kg.	Hematocrit Per Cent. Plasma	Blood Volume	C.c. per Kg.	Per Cent. Body Weight	Fraction Body Weight	Remarks
None	100/ 60	None	1,850	50						
None	120/ 80	None	3,750	53	60	6,250	89	9.3	1/10.9	6 feet 3 inches in height
None	120/ 70	None	2,725	28.6						
Slight	210/110	Moderate	4,950	45.8						
None	100/ 60	None	2,575	66	75	3,425	88	9.2	1/10.8	
None	150/100	None	2,375	45						
Right leg sacrum	130/ 65	None	3,150	64	77.2	4,075	84	8.8	1/11.4	
None	100/ 65	None	2,175	49	65.3	3,325	76	8	1/12.5	
None	110/ 65	Slight	3,725	66						
Moderate	105/ 45	Slight	3,425	60	85.6	4,025	70	7.3	1/13.6	
None	.....	None	2,900	57	78.8	3,675	72	7.5	1/13.2	
None	120/ 90	Slight	2,120	47.6	67.3	3,150	71	7.4	1/13.5	
None	100/ 65	Slight	3,400	57	70.6	4,800	88	9.2	1/10.8	
None	120/ 75	Slight	3,225	51	64.3	5,025	80	8.4	1/12	
None	100/ 55	None	4,950	72	90	5,500	81	8.5	1/11.7	
None	120/ 70	Slight	3,450	54	84	4,250	66	6.9	1/14.4	Improved
None	.....	None	2,850	53	.....	.....	...	....	.....	Died
None	120/ 70	None	1,975	39	53.2	3,725	73	7.7	1/13.5	
.....	.....	.....	2,000	39	53.2	3,750	73	7.7	1/13.5	
None	135/ 90	Slight	4,125	59.7	52.5	7,880	113	11.9	1/8.4	
None	140/ 90	None	2,175	51.3	57	3,800	89	9.3	1/10.7	Blood glucose 0.25-0.3 Necropsy
None	160/ 60	Slight	3,275	56	62.1	5,375	92	9.6	1/10	Blood glucose 0.45-0.18
None	120/ 70	None	2,825	35	.....	.....	...	....	.....	Blood glucose 0.18
None	120/ 80	None	2,775	51	.....	.....	...	....	.....	Blood glucose 0.4-0.34
Marked	90/ 50	Moderate	3,900	50						
Marked	120/ 80	Marked	4,375	50	54.3	8,050	92	9.6	1/10.3	Necropsy
Slight	.....	Marked	3,900	53						
Moderate	170/120	Moderate	3,800	42.5						
Moderate	130/ 90	Marked	3,025	40	45.6	6,650	87	9.1	1/11	
.....	.....	.....	3,175	43						
Moderate	150/115	Marked	3,975	43	.....	.....	...	....	.....	Necropsy
Slight	130/ 90	Slight	3,385	56	.....	.....	...	....	.....	Necropsy
.....	115/ ?	.....	2,841	48						
Slight	160/ 90	Slight	3,300	36.7	.....	.....	...	....	.....	Weight 130 lbs. in 1903

TABLE 8.—Clinical (Pathological)—

No.	Name	Date	History No.	Age	Diagnosis	Red Blood Cells	Hgb. Sahli.	Weight	
								Actual	Best
32	L. J.	....	33777	47	Hypertension. Chronic nephritis. Osteo-arthritis of spine	5,000,000	90	79.4	...
33	E. F.	1/20	33757	36	Syphilis. Arteriosclerosis. Hypertension. Chronic nephritis	3,500,000	65	62.3	65
		3/18	.....	..	.....	.....	..	.....	...
34	E. O.	2/21	33697	47	Arteriosclerosis. Chronic nephritis. Hypertension. Polycythemia	5,900,000	105	72	80
		2/27	.....	..	.....	.....	..	.....	...
35	G. L.	1/20	33510	53	Syphilis. Hypertension	5,000,000	83	60.7	70
36	R. A.	2/17	33643	45	Chronic nephritis. Hypertension. Myocardial insufficiency	3,000,000	60	65.9	...
37	F. W.	2/17	33722	47	Chronic nephritis. Hypertension. Cerebral hemorrhage	5,800,000	92	56.6	...
38	M. C.	1/23	C. H. I.	..	Hypertension	.....	..	42.7	...
39	J. E. A.	2/12	36283	49	Chronic nephritis. Hypertension. Arteriosclerosis. Hematuria	a. m.,		78.3	...
		2/18				4,000,000; p. m., 3,700,000	80	76.4	...
							75	.....	...
		2/25	.....	..	.....	.....	..	75.9	...
40	H. O. C.	1/14	36082	55	Arteriosclerosis. Hypertension. Enlarged prostate	.....	..	70.1	...
41	N. P. P.	3/18	36551	51	Arteriosclerosis. Hypertension. Carcinoma of bladder	3,800,000	72	98.4	...
42	E. S. H.	2/12	36298	71	Arteriosclerosis. Hypertension ..	.....	..	59.1	...
43	M. P.	1/12	33549	32	Chronic nephritis. Hypertension. Myocardial insufficiency	4,400,000	63	87.3	...
		2/3	.....	..	.....	.....	..	76.3	...
44	W. L. I.	1/12	33424	38	Chronic nephritis	4,000,000	82	80.9	...
		2/3	.....	..	.....	.....	..	79.5	...
45	T. K.	1/12	33544	29	Chronic nephritis	4,200,000	93	62.7	73
46	A. T.	1/3	33472	22	Chronic prostatitis	4,300,000	100	52.3	61
47	T. W.	1/7	33479	71	Chronic infectious arthritis.....	4,800,000	75	72.7	73
48	D. K. S.	1/12	33572	51	Cerebral arteriosclerosis. Brain tumor?	.....	..	56.4	...
49	F. N.	1/20	33390	40	Emphysema. Chronic bronchitis	5,000,000	90	54.8	...
50	B.	1/7	33333	43	Syphilis. Aneurysm of thoracic aorta	4,200,000	92	61.4	...
		2/2	.....	..	.....	.....	..	63.6	...
51	F. T.	1/14	33545	53	Chronic appendicitis	5,000,000	90	48.6	...
52	M.	1/14	33461	18	Typhoid fever	4,500,000	85	60	...
53	S. B.	2/27	36426	49	Carcinoma bladder. Secondary anemia	3,300,000	33	58	...
		3/11	.....	..	.....	.....	..	58.4	...

STUDIES ON BLOOD VOLUME—(*Continued*)

Edema	Blood Pressure	Cardiac Enlargement	Plasma c.c.	C.c. Per Kg.	Hematocrit Per Cent. Plasma	Blood Volume	C.c. per Kg.	Per Cent. Body Weight	Fraction Body Weight	Remarks
Slight	180/100	Moderate	2,700	34	66.7	4,050	51	5.3	1/18.7	
None	200/150	Slight	3,050	49						
.....	.....	.....	3,150	51						
None	200/150	Slight	3,050	42	56.1	5,475	76	8	1/12.5	Blood glucose 0.12
.....	.....	.....	.....	48	61	5,675	78	8.2	1/12	
None	185/105	Slight	3,025	50						
Slight	240/130	Slight	3,175	48	63	4,913	74	7.8	1/12.7	Blood glucose 0.14. Died
None	195/130	Slight	2,950	52	54.3	5,425	96	10	1/10	
None	180/ ?	Slight	1,825	43						
None	245/140	Moderate	4,650	59						
.....	235/135	.....	3,875	51	63	6,150	84	8.8	1/11.9	800 c.c. blood removed
.....	235/155	.....	3,425	45	63	5,425	80	8.4	1/13.1	
.....	220/125	.....	4,150	55	75	5,500	72	7.6	1/13.1	
None	175/110	Slight	3,500	50	60.8	5,750	81	8.5	1/11.7	
None	195/110	Slight	4,325	44	66.4	6,525	65	6.8	1/14.7	Died
None	210/ 90	Moderate	3,150	53						
Considerable	180/100	Marked	3,875	44	64.4	6,025	69	7.2	1/13.9	
Slight	115/ 75	Slight	4,047	53						
Moderate	130/ 80	Slight	3,172	39						
.....	.....	.....	3,064	38.4						
None	100/ 85	None	2,925	46						
None	140/ 90	Slight	2,420	45						
None	140/ 85	None	3,450	47.6	63.7	5,425	75	7.8	1/12.8	
None	120/ ?	Slight	2,825	56.4						
None	110/ 70	None	2,450	45						
None	120/ 70	Slight	3,025	49	60	4,900	78	8.1	1/12.2	
.....	.....	.....	3,175	50						
None	110/ 70	None	2,200	46						
None	95/ 60	None	3,050	50						
None	168/ 88	Slight	3,500	61	70.6	4,550	78	8.2	1/12.2	Died
.....	.....	.....	3,350	57						

Although in anasarca the number of cubic centimeters of blood per kilogram may not be increased above normal, the total blood volume may be large in relation to the normal body weight. Case 25 had a blood mass of over 8 liters. With the disappearance of his edema, he lost 14 kg. Considering his weight at this time in relation to the blood volume, 8,050 c.c., found during edema, he had 109 c.c. per kilogram, which is an extremely high value. The patient died six weeks later. At necropsy, the kidney showed numerous small scars throughout, with some atrophy of the tubular cells, and also an acute inflammatory process, as evidenced by infiltration with polymorphonuclear leukocytes. There was a slight chronic nephritis with an acute terminal process. Myocarditis was extensive and fibrous in type, with hypertrophy of muscle and small scattered scars everywhere. The heart was of tremendous size. It is quite possible that in this instance the large blood mass stood in causal relationship to the large heart.

Hypertension and Chronic Nephritis.—In nineteen determinations on thirteen cases (Nos. 31 to 43, inclusive, Table 8), a plasma volume greater than normal was found only once, while the total blood volume was not found increased in nine determinations on six cases. This would appear to demonstrate that in these cases the hypertension was not dependent on an increase in blood mass. In hypertension, the blood volume is normal and frequently low. The increased tension would appear, therefore, to be dependent on a vascular system that is too small, rather than on a blood volume that is too large. The average value for plasma was 42.8 c.c. per kilogram, and for the whole blood, 75 c.c. per kilogram, in both instances the lower limit of normal. The small values are not dependent on edema, as will be ascertained from the table, since in only three cases was even slight edema present, and in only one was there great cardiac enlargement. The results are in striking contrast with those of Plesch.[14]

Several of these cases are of peculiar interest. Patient 39, who, on one determination showed an increased plasma volume, had a marked hematuria so that hemorrhage may have accounted for this finding. Subsequent determinations showed the plasma volumes to be normal. Following venesection with removal of 800 c.c., there was no decrease in blood pressure, but immediate decrease in plasma volume. A week later, there was an increase in plasma to above that found before the venesection, but without a corresponding increase in the blood volume.

That hypertension can exist in association with a low blood volume is clearly indicated by Case 32, the patient showing a systolic blood pressure of 180 and diastolic of 100. The plasma was 34 c.c. per kilogram and the total blood, 51 c.c. per kilogram. The hypertension

existed despite the fact that the patient's total blood mass was not greater than the plasma volume is normally. Obesity and a slight edema were in part responsible for these low values.

Case 43 is also of great interest. The patient is the only one of the series presenting marked edema at the time of determination. At this time, he had 40 c.c. of plasma per kilogram. Following a loss of 11 kg. (disappearance of edema), the plasma volume was found to be slightly larger, 4,050 c.c., compared with 3,875 c.c. It is evident that the plasma volume remained the same throughout, not being influenced by the presence or disappearance of the edema. This does not always occur, as will be seen by contrasting with Case 25.

From these cases, it would appear that true plethora is not present in hypertension, and that a small or contracted vascular system and not an increase in blood mass is responsible for the increased blood pressure.

Chronic Nephritis Without Hypertension.—Only two cases (Nos. 44 and 45, Table 8) fall into this group. Patient 44 was an instance of chronic parenchymatous nephritis, with slight edema and very marked albuminuria. The plasma was estimated twice, three weeks intervening. Following a 1.5 kg. decrease in weight, the plasma decreased 100 c.c. Both cases gave values below the normal average for plasma.

Miscellaneous Group.—A small series of miscellaneous unrelated cases (Nos. 46 to 53, inclusive, Table 8) were studied. Case 50, an instance of aneurysm of the ascending arch of the aorta, and with a moderate grade of anemia, showed identical results on two occasions—an average normal value for the plasma. Patients 49, suffering with chronic bronchitis; 51, with chronic appendicitis; 46, a neurasthenic, and 48, with cerebral arteriosclerosis, all showed values within the limits of normal.

COMMENT

Except in pregnancy, hypertension and obesity, the number of cases studied in any one condition has been so small that far-reaching conclusions are impossible. Sufficient evidence has been adduced to convince us of (*a*) an increase in blood volume just before term in uncomplicated pregnancy, (*b*) absence of a causal relationship between increased blood volume and hypertension, and (*c*) of a relatively small blood mass in obesity. Further blood volume studies in these and in other clinical conditions is contemplated.

From this study, it is apparent that a great variation in the blood mass in relation to body weight may occur in any one disease. Complications of any kind may also exert a great influence on the blood volume, as evidenced by Case 2 in Table 7. It becomes evident that

we cannot take for granted that the blood volume is increased or decreased in any given disease, but must actually determine it in each individual case. In the present state of the subject, generalizations are undesirable.

SUMMARY

1. A method for the determination of plasma volume is described. From the plasma volume and hematocrit values the blood volume can be calculated.

2. With this method, duplicate determinations on normal subjects yield identical values.

3. The amount of decrease in blood volume as the result of hemorrhage and of increase following intravenous infusion of saline has been demonstrated.

4. Changes in blood volume are insignificant in experimentally induced hypertension and hypotension, and cannot be brought into causal relationship with them.

5. The plasma normally constitutes approximately 5 per cent. or one-twentieth of the body weight. Normal individuals have approximately 50 c.c. of plasma per kg., the extremes being from 42 to 56 c.c. per kilogram.

6. The normal hematocrit values obtained were approximately 43 per cent. for erythrocytes and 57 per cent. for plasma.

7. The blood normally constitutes 8.8 per cent., or 1/11.4 of the body weight. Normal individuals have approximately 85 c.c. of blood per kilogram, the extremes being 78 and 97 per kilogram.

8. In pregnancy, before term, the blood and plasma volumes are increased. A condition of serous plethora exists. Within a week or two after delivery, the blood values return to normal.

9. In obesity, the plasma and blood volumes are relatively small.

10. Many cases of anemia exhibit a relatively large plasma volume.

11. Polycythemia in the sense of a high blood count may be dependent on a low plasma volume. It may be associated with a large plasma volume, in which case true plethora is indicated.

12. Hyperglycemia exists without increase in blood volume.

13. In anasarca accompanying myocardial insufficiency the blood volume may be absolutely increased.

14. A small volume is shown in many cases of marked hypertension. Therefore so far as these studies go it would appear that hypertension is not dependent on a large blood volume.

STUDIES OF NITROGEN PARTITION IN THE BLOOD AND SPINAL FLUID

WITH ESPECIAL REFERENCE TO THE POSSIBLE CAUSATION OF ALBUMINURIC RETINITIS *

ALAN C. WOODS, M.D.
BOSTON

The fact that an albuminuric retinitis, occurring in the course of a chronic nephritis, is a sign of the gravest prognostic import, has long been recognized. Within the last five years, this has been the subject of considerable investigation, especially by the French clinicians, and several very interesting theories have been advanced. Chauffard[1] attempted to explain albuminuric retinitis on the grounds of a hypercholesterinemia, while Onfrey and Balavoine[2] attempted to show that changes in the viscosity of the blood played an important part. The theory that attracted the greatest amount of attention, however, was that advanced by Widal[3] in 1910. He stated that albuminuric retinitis was the result of the retention in the blood of urea, or of some nitrogenous body closely allied to urea. In 1912 Widal[4] reviewed the work of Chauffard and agreed with him that cholesterin, lipoids, and lecithin compounds might often be retained in the blood in nephritis. Widal found, however, that this retention fell as the urea retention rose and stated that retinitis occurred only in those cases which showed a definite nitrogen retention. The prognostic significance of albuminuric retinitis was due, therefore, he believed, to the fact that it stood as a sign of nitrogen retention.

The object of my work has been to determine whether such a relation between nitrogen retention and the occurrence of albuminuric retinitis could be demonstrated, and whether or not albuminuric retinitis could be charged to "urea or to any closely allied nitrogenous body." Using the newer methods, estimations were made of the total nonprotein nitrogen, of the ammonia nitrogen, of the urea nitrogen, the

* Submitted for publication May 4, 1915.
* From the Medical Clinic of the Peter Bent Brigham Hospital.

1. Chauffard: Pathogénie des rétinites albuminuriques, Semaine méd., 1912, xxxii, 193.
2. Onfrey and Balavoine: Viscosité du sang et hémorragies oculaires, Ann. d'ocul., 1911, cxlvi, 433.
3. Widal, Morax and Weill: Rétinite Albuminurique et Azotémie, Ann. d'ocul., 1910, cxliii, 354.
4. Widal, Weill and Landat: La lipémie des brightiques; rapports de la rétinite des brightiques avec l'azotémie et la cholesterinémie, Semaine méd., 1912, xxxii, 529.

uric acid, creatinin, combined creatinin and creatin, and of the amino-acid nitrogen in the blood. Estimations were also made of the total nonprotein nitrogen and urea nitrogen in the spinal fluid, taken at the same time.

In a review of the literature, the first important work on nonprotein nitrogen retention in the blood seems to have been done by von Jaksch[5] in 1893. He gave figures for the nitrogen content of the alcoholic filtrate from blood, and showed there was a marked increase in the nonprotein nitrogen in the blood in chronic nephritis. H. Straus[6] in 1902 gave figures in nitrogen partition, finding 75 per cent. of the total nonprotein nitrogen as urea nitrogen, 2.4 per cent. as uric acid, and 5 per cent. as ammonia. In high nitrogen retention, he found these same figures held. Holweg[7] in 1911, found 60.8 per cent. of the total nonprotein nitrogen as urea nitrogen, 27.4 per cent. as amino-acid nitrogen and 11.7 per cent. as albuminose nitrogen. He found that as the total nonprotein nitrogen retention increases, the urea and amino-acid nitrogen increased proportionally, while the albuminose nitrogen remained stationary. Obermayer and Popper[8] in 1911 reviewed the literature and cited the following results of investigation on the retention of nitrogenous bodies. Jaccoud[9] thought creatin retention the cause of uremia. Coetbeer and Hunden[10] found that in the great nonprotein nitrogen retention following extirpation of the kidney, urea nitrogen was only one-third the total. Oppler and Hoppe-Seyler[11] found creatinin increased in the blood in chronic nephritis, and von Jaksch[12] found an increase in uric acid. Obermayer and Popper themselves found nonprotein nitrogen in the blood in cases of uremia varying from 57 to 658 mg. per hundred c.c. of blood, of which an average of 64 per cent. was urea nitrogen.

Philipp[13] in 1913 reported a series of cases giving the total nonprotein nitrogen and the urea nitrogen content in the blood in cases of uremia. Using phosphotungstic acid to precipitate the proteins in the determination of the total nonprotein nitrogen, he found the urea

5. Von Jaksch: Ueber die Zusammensetzung des Blutes gesunder und kranker Menschen, Ztschr. f. klin. Med., 1893, xxiii, 187.

6. Straus, H.: Die chronischen Nierenentzündungen in ihrer Einwirkung auf Blutflüssigkeit, Berlin, 1902.

7. Holweg: Ueber das Verhalten des Reststickstoffes des Blutes bie Nephritis und Urämie, Deutsch. Arch. f. klin. Med., 1911, civ, 216.

8. Obermayer and Popper: Ueber Urämie, Ztschr. f. klin. Med., 1911, lxxii, 332.

9. Jaccoud: Quoted by Obermayer and Popper, Note 8.

10. Soetbeer and Hunden: Quoted by Obermayer and Popper, Note 8.

11. Oppler and Hoppe-Seyler: Quoted by Obermayer and Popper, Note 8.

12. Von Jaksch: Quoted by Obermayer and Popper, Note 8.

13. Philipp: Ueber des Verhalten des Harnstoffs und des Reststickstoffs im Blute von Nephritiken, med. Klin., Berlin, 1913, ix, 912.

nitrogen as an average of 21.2 per cent. of the total nonprotein nitrogen. The highest total nonprotein nitrogen he reported was 289 mg. per hundred c.c. of blood in a case of mercury poisoning. Agnew[14] in 1914 reports a series of estimations of total nonprotein nitrogen and urea nitrogen.

The more recent work on the subject of nitrogen partition has been done by Folin and his co-workers. In 1913, Folin and Denis[15] reported on the determination of nitrogenous waste products, and stated that they had found no particular diagnosis to correspond with any particular degree of nitrogen retention. At the same time Seymour[16] reported a series of determinations of total nonprotein nitrogen, urea nitrogen, and uric acid in the blood. In 1914, Folin and Denis[17] reported further on the influence of diet on these same nitrogenous bodies. Again in 1914, Folin and Denis[18] reported a series of twenty-seven estimations of the total nonprotein nitrogen, ammonia nitrogen, uric acid, creatinin, and combined creatinin and creatin, in cases both of health and disease. In four estimations on healthy individuals, the total nonprotein nitrogen varied from 24 to 37 mg. per hundred c.c. of blood, urea nitrogen from 11 to 18 mg.; ammonia nitrogen from 0.1 to 0.14 mg.; uric acid from 2 to 3 mg.; creatinin from 1.1 to 1.3 mg. and combined creatinin and creatin from 6.5 to 9.5 mg. per hundred c.c. of blood. In pathologic conditions, figures were found as high as 326 mg. for the total nonprotein nitrogen in a case of decompensated cardiorenal disease. In uremia the highest figures found for the various nitrogenous bodies were, total nonprotein nitrogen 264 mg., urea nitrogen 228 mg., ammonia nitrogen 0.66 mg., uric acid 6.6 mg., creatinin 26 mg., and combined creatinin and creatin 46 mg., all per hundred c.c. of blood. In 1915 Foster[19] reported he had found substantially the same figures in

14. Agnew, J. Howard: Comparative Study of Phenolsulphonephthalein Elimination, and the Incoagulable Nitrogen in the Blood in Cardiorenal Diseases, The Archives Int. Med., 1914, xiii, 485.

15. Folin, Otto, and Denis, W.: Nitrogenous Waste Products in the Blood in Nephritis, Their Significance and the Methods of Determination, Medical Communications, Massachusetts Med. Soc., 1913, xxiv, 157.

16. Seymour: Nitrogenous Waste Products in the Blood, Their Effect in Chronic Interstitial Nephritis, Medical Communications, Massachusetts Med. Soc., 1913, xxiv, 163.

17. Folin, Otto, Denis, W., and Seymour, Malcolm: The Nonprotein Nitrogenous Constituents of the Blood in Chronic Vascular Nephritis (Arteriosclerosis) as Influenced by the Level of Protein Metabolism, The Archives Int. Med., 1914, xiii, 224.

18. Folin, Otto, and Denis, W.: On the Creatinin and Creatin Content of Blood, Jour. Biol. Chem., 1914, xvii, 487.

19. Foster, Nellis B.: Uremia. The Nonprotein Nitrogen of Blood, The Archives Int. Med., 1915, xv, 356.

nitrogen partition as had Folin. In addition, four estimations of the amino-acid nitrogen gave values between 4 and 6 mg. per hundred c.c. of blood.

Nitrogen studies in the spinal fluid have been confined largely to estimations of the urea content. The literature was reviewed in 1909 by Mollard and Froment,[20] and brought up to date in 1914 by Soper and Granat.[21] Figures given by various investigators for the normal urea content vary from 0 to 40 mg. per hundred c.c. of spinal fluid, but practically all authors agree that it is greatly increased in uremia. Mestrezat[22] in 1912 gives the normal urea content as 6 mg. and the total nonprotein nitrogen as 16.7 mg. per hundred c.c. of spinal fluid. Galletta[23] in 1908 gave the total nonprotein content as 20.6 mg., and Comba[24] in 1899, as 15 mg. per hundred c.c. of spinal fluid. Both authors cite cases of uremia in which the nonprotein nitrogen retention is vastly increased. Widal[25] and Javal[26] in 1911 showed that the concentration of urea in the various body fluids is substantially the same. Soper and Granat[21] in 1914 reported a series of urea estimations in the spinal fluid, using the hypodermic methods, and emphasized the prognostic significance of these. Moral and Froment[27] in 1913 reported a case of amyloid kidney, in which they found a total nonprotein nitrogen retention of 222 mg., per hundred c.c. of spinal fluid, of which 220 mg. were represented as urea nitrogen.

In my series of cases, about 175 c.c. of blood were received directly from the vein into a cylinder containing 3 c.c. of a 20 per cent. solution of potassium oxalate. The cylinder was rapidly shaken to prevent clotting. Lumbar puncture was done immediately thereafter, and about 10 c.c. of spinal fluid withdrawn.

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20. Mollard and Froment: Urée dans le liquide céphalo-rachidien et urémie nerveuse, Jour. de Physiol. et de Path. Général, 1909, xi, 263.

21. Soper, Willard B., and Granat, Selma: The Urea Content of the Spinal Fluid with Special Reference to Its Diagnostic and Prognostic Significance, The Archives Int. Med., 1914, xiii, 131.

22. Mestrezat: Le Liquide Céphalo-Rachidien, Paris, 1912.

23. Galletta: Quoted by Mestrezat, Note 22.

24. Comba: Quoted by Mestrezat, Note 22.

25. Widal: Le prognostic dans le mal le Bright par le dosage de l'urée du sang. Les rémissions temporaires et trompeuses de l'azotémie, Soc. med. d. hôp. de Paris, 1911, xxxii, 627.

26. Javal: La grande azotémie. Ses formes, son évolution, son prognostic étudiés par le dosage méthodique de l'urée dans le sang et dans les sérosités de l'organisme, Soc. med. d. hôp. de Paris, 1911, xxxii, 485.

27. Moral and Froment: A propos d'un cas de néphrite amyloide; rétention de l'urée dans le liquide céphalo-rachidien, Lyon méd., 1913, cxx, 933.

In all estimations on the blood, whole blood was used. Total nonprotein nitrogen,[28] ammonia nitrogen,[28] creatinin,[29] and combined creatin and creatinin[29] were done by the methods of Folin and Denis. Uric acid was determined by the method of Folin and Denis,[30] using the later modification of Folin and Bell,[31] in which sodium thiocyanate is used in place of hydrogen sulphid. Urea nitrogen was determined by the method of Van Slyke,[32] using the colorimeter. The amino-acid nitrogen was estimated with Van Slyke's micro-apparatus.[33] Chlorids were estimated by the method of McLean.[34] Total nonprotein nitrogen and urea nitrogen determinations in the spinal fluid were done synchronously with the determinations in the blood. All results were controlled and were repeated when the difference between the estimations was appreciable. The systolic and diastolic blood pressures were taken just before bleeding, and phenolsulphonephthalein tests were done in all instances. The creatin was estimated as the difference between the combined creatinin and creatin determination, and the creatinin determination. The uric acid, creatinin, and creatin nitrogen were figured from the structural formula of each, and are reported as mg. per hundred c.c.

The cases are reported in three groups: (1) those cases which presented no retinal changes whatsoever; (2) those cases which presented the retinal picture of arteriosclerosis, with occasional hemorrhages, but without exudates; (3) those cases which showed a marked albuminuric retinitis, with haziness or obliteration of the disk outlines, vascular changes, hemorrhages and exudates, with involvement of the macula region.

While the highest retention observed, 266 and 257 mg. of nonprotein nitrogen per hundred c.c. of blood, happened to occur in cases of albuminuric retinitis, nevertheless marked retinitis occurred at practically any level of nitrogen retention. There appears to be no close relationship whatsoever between either total nonprotein nitrogen retention, or any component part, and the occurrence of albuminuric retinitis. A comparison of the three groups fails to show any appreciable

28. Folin, O., and Denis, W.: New Methods for the Determination of Total Nonprotein Nitrogen, Urea and Ammonia in the Blood, Jour. Biol. Chem., 1912, xi, 527.

29. Folin, O., and Denis, W.: On the Determination of Creatinin and Creatin in Blood, Milk and Tissues, Jour. Biol. Chem., 1914, xvii, 475.

30. Folin, O., and Denis, W.: A New (Colorimetric) Method for the Determination of Uric Acid in Blood, Jour. Biol. Chem., 1912-1913, xiii, 469.

31. Courtesy of Drs. Folin and Bell. Method as yet unpublished.

32. Van Slyke and Cullen: A Permanent Preparation of Urease and Its Use in the Determination of Urea, Jour. Biol. Chem., 1914, xix, 221.

33. Van Slyke and Meyer: The Amino-Acid Nitrogen of the Blood, Jour. Biol. Chem., 1912, xii, 399.

34. Courtesy of Dr. McLean. As yet unpublished.

difference in the proportions of either urea, ammonia, uric acid, creatinin, creatin, or amino-acid nitrogen. The same proportions prevail in cases in which there is albuminuric retinitis and those in which the fundi are normal. Neither to urea nor to "any closely allied nitrogenous body" can albuminuric retinitis be attributed. This holds true for the cerebrospinal fluid, also, so far as nitrogen retention could be studied. The three groups show essentially the same picture. Case 13 was of especial interest in that the patient presented a fresh albuminuric retinitis together with a normal nitrogen content throughout. He was a man of 52 years, admitted in apoplectic coma, with a systolic blood pressure of 285 mm. Hg, marked retinitis and a phenolsulphonephthalein output of 28 per cent. He made a good recovery and was discharged from the hospital in fair condition.

An analysis of the figures shows that the urea maintains roughly the same concentration as the total nonprotein nitrogen rises, an average throughout of 63.4 per cent. of the total nonprotein nitrogen. While the ammonia nitrogen increased slightly as the total nonprotein nitrogen rose, this rise was not proportional. The relation of ammonia nitrogen to total nonprotein nitrogen accordingly varied from about 0.4 per cent. at the low levels of total nonprotein nitrogen to about 0.1 per cent. at the highest levels. The uric acid figures were the last to show any marked increase as the total nonprotein nitrogen rose. But two cases showed any marked uric acid retention—Nos. 9 and 17B, in which the uric acid content was respectively 5 and 8.35 mg. per hundred c.c. of blood. In these two cases blood was taken 36 and 12 hours before death. The creatinin showed roughly a proportionate increase as the total nonprotein nitrogen rose, averaging in all 3.4 per cent of the total nonprotein nitrogen. The creatin percentage, excluding Case 18, varied from about 2 per cent. to 7 per cent. throughout, averaging 3.9 per cent. In Case 18 it reached the high value of 12 per cent. The amino-acid nitrogen, however, showed no constant increase as the total nonprotein nitrogen rose to higher levels, remaining generally between 4 and 7 mg. per hundred c.c. of blood. In Cases 9, 10, 15B and 16A it reached higher levels, but in each of these cases there was an extraneous factor. Case 9 was one of complete anuria for seven days, the blood being taken on the fifth day, while in Cases 10, 15B and 16A the blood was taken about one hour after the midday meal. In all other cases blood was taken four hours after the midday meal.

In the limited number of cases in which as complete partitions as possible were done, figures are given showing the "residual nitrogen." These figures were obtained by subtracting the sum total of the determined component nitrogen constituents from the total nonprotein

nitrogen. It includes, therefore, the total error of all the various estimations, and such other unknown nitrogenous bodies as may enter the blood stream. These figures are of interest in that they seem in several cases to run roughly parallel with the severity of the case as observed clinically. In Case 9, in which the high figure for "residual nitrogen" of 31.55 mg. per hundred c.c. of blood was reached, after five days of complete anuria, death occurred in thirty-six hours. In Cases 7, 8 and 16, in which the values were all over 10 mg. per hundred c.c. of blood, death occurred in two days, one month and two months respectively. In Cases 11 and 12 with high figures for residual nitrogen, the prognosis from the clinical standpoint seemed extremely poor. In the remaining cases in which residual nitrogen was estimated, the patients were all in reasonably fair condition.

The total nonprotein nitrogen in the spinal fluid was, as a rule, about 25 per cent. lower than that in the blood. In two cases, however, the total nonprotein nitrogen was the same in both. The first of these (No. 8) was one of mercurial poisoning, with practically complete anuria for five days previous to death. Blood and spinal fluid were taken eighteen hours before exitus. The figures here are comparatively low when compared to Philipp's case of mercurial poisoning, which showed a nonprotein nitrogen retention of 289 mg. The second of these two cases was one of seven days' complete anuria and the blood and spinal fluid were taken on the fifth day. It seems possible that certain nitrogenous bodies, normally not present in the spinal fluid, are, in anuria, excreted from the blood into the spinal fluid until an equilibrium is reached. Sufficient spinal fluid was never obtained in any case to determine if these bodies were any of the common nitrogenous constituents of the blood, for example, uric acid, creatinin, creatin or amino-acids. The urea content of the spinal fluid throughout approximately equaled that of the blood, as shown before by Widal, Javal and others. It does not seem probable that nitrogen determinations in the spinal fluid give any greater diagnostic or prognostic significance than those in the blood.

The concentration of urea in the spinal fluid, in relationship to the total nonprotein nitrogen, was fairly constant throughout, averaging approximately the same percentage at high as at low levels, an average of 78 per cent. in the series as a whole.

The chlorids in the blood showed little variation, varying in most cases between 0.42 and 0.53 gm. per hundred c.c. In the case of mercury poisoning the low figure 0.34 gm. per hundred c.c. was reached. Isolated determinations of blood chlorids without taking into consideration the relative intake and excretion, give little or no information about salt retention.

TABLE I.—Chronic Nephritis with Normal Fundi

No.	Hosp. Med. No.	Blood Nitrogen									Spinal Fluid Nitrogen		Blood Pressure	Phthalein Excretion Percentage in 2 Hrs.	Remarks
		Total Non-protein	Urea	Ammonia	Uric Acid	Creatinin	Creatin	Amino-Acid	"Residual"	Chlorids, Gm.	Total Non-protein	Urea			
1	2,358	26.77	11.38	0.09	0.40	0.77	0.99	6.78	3.36	0.4476	17.85	11.09	130- 85	48	Normal control
2	2,319	29.43	15.63	0.15	0.34	0.57	0.65	5.42	6.67	0.4435	24.68	15.45	240-160	47	
3	2,357	35.70	18.49	0.10	0.67	0.67	0.95	8.44	6.48	0.4391	27.35	18.50	210-108	52	Chr. myocarditis
4	2,348	43.48	23.57	0.12	0.40	0.59	1.19	6.52	11.09	0.4707	35.70	21.25	185- 95	52	
5	1,992	55.30	38.98	0.14	0.67	3.07	4.27	....	....	0.5153	.....	.....	260-110	12	
6	1,954	57.60	36.85	0.15	0.64	0.82	5.70	....	....	0.4296	.....	.....	235-115	21	
7A†	2,006	95.27	66.66	0.15	0.64	3.68	2.89	....	....	0.5363	.....	.....	190- 98	Unreadable trace	
7B‡		90.90	62.50	0.19	0.53	5.08	2.32	6.42	13.86	0.5682	66.66	60.24	190-100	Unreadable trace	
8	2,259	128.00	95.10	0.16	0.68	5.29	7.08	5.89	13.80	0.3429	128.00	95.21	130- 80	.....	Mercury poisoning
9	2,174	138.10	79.76	0.24	1.67	4.09	4.37	16.32	31.56	0.4216	142.96	85.88	185-140	Unreadable trace*	

* Phthalein estimation made three weeks previously at Massachusetts General Hospital. † 7A on December 12. ‡ 7B on December 17

TABLE 2.—Chronic Nephritis with Vascular Changes Only in Fundi

No.	Hosp. Med. No.	Blood Nitrogen									Spinal Fluid Nitrogen		Blood Pressure	Phthalein Excretion Percentage in 2 Hrs.	Remarks
		Total Non-protein	Urea	Ammonia	Uric Acid	Creatinin	Creatin	Amino-Acid	"Residual"	Chlorids, Gm.	Total Non-protein	Urea			
10	2,132	47.51	29.58	0.18	0.40	2.02	3.58	10.23	1.52	0.4500	.....	.....	265-140	33	
11	2,202	71.34	43.07	0.11	0.66	1.71	2.10	6.50	17.28	0.5362	47.62	40.32	245-150	18	17 cells in sp. fl.
12	2,316	95.23	60.00	0.16	0.43	3.68	4.34	8.05	18.57	0.4649	80.00	60.16	213-124	Unreadable trace	

TABLE 3.—Chronic Nephritis with Marked Albuminuric Retinitis

No.	Hosp. Med. No.	Blood Nitrogen									Spinal Fluid Nitrogen		Blood Pressure	Phthalein Excretion Percentage in 2 Hrs.	Remarks
		Total Non-protein	Urea	Ammonia	Uric Acid	Creatinin	Creatin	Amino-Acid	"Residual"	Chlorids, Gm.	Total Non-protein	Urea			
13	2,354	23.81	14.60	0.10	0.35	0.61	0.99	4.02	3.14	0.4243	20.00	12.50	285-160	28	Apoplexy
14	2,074	40.98	27.00	0.17	0.39	1.67	2.21	5.22	4.32	0.4400	33.28	26.32	180-130	19	
15A†	2,043	54.05	32.14	0.12	0.46	2.74	2.74	....	....	0.4558	43.46	26.59	250-166	10	
15B‡		60.61	41.54	0.12	1.26	2.30	3.12	10.64	1.63	0.4735	46.58	31.25	250-160	10	
16A‖	2,108	55.55	29.35	0.09	0.60	2.63	1.59	9.44	11.85	0.4407	37.21	27.77	190-150	15	Four cells per cm. in spinal fluid
16B¶		47.54	25.50	0.15	0.98	2.45	3.66	6.56	8.24	0.3997	33.33	26.00	150-130	11	Herpes zoster complicating uremia. Ninety-one cells
17A#	1,882	192.30	113.80	0.20	0.62	8.01	14.39	....	....	0.4788	125.00	111.11	248-128	Unreadable trace	Sixteen cells per cm. spinal fluid
17B§		266.66	166.45	0.21	2.78	7.85	7.66	....	....	0.5141	188.69	166.66	250-130	Unreadable trace	Uremia. Twenty-three cells per cm. in spinal fluid
18	1,975	257.70	181.80	0.33	1.51	6.01	30.93	....	....	0.4752	.....	.....	208-152	Unreadable trace	Uremia

† 15A on December 19. ‡ 15B on December 30. ‖ 16A on January 4. ¶ 16B on January 19. # 17A on November 30. § 17B on December 14.

The relationship between nonprotein nitrogen retention and phenolsulphonephthalein excretion was fairly constant. When the nitrogen retention was high, the phenolsulphonephthalein excretion was generally low, and vice versa, confirming the results of the numerous investigators in this field. No relationship between the nonprotein nitrogen retention and blood pressure was observed.

CONCLUSIONS

1. There is no apparent relationship between the retention of any nitrogenous body and the occurrence of albuminuric retinitis, and there is certainly no evidence that they stand in the relationship of cause and effect.

2. In the limited number of cases in the series, there seemed to be a more than casual relationship between the "residual nitrogen" of the blood, and the severity of the case as observed clinically.

3. As the level of nonprotein nitrogen rises, the component nitrogenous bodies increase, and in the case of the chief constituents, this rise is proportioned.

4. Estimations of total nonprotein nitrogen and of urea in the spinal fluid give no greater diagnostic or prognostic significance than estimations of these substances in the blood.

5. The variations in chlorid concentration in the blood are so small that in themselves they give no idea of salt retention.

I wish to express my thanks to Dr. Henry A. Christian for his advice and for facilities placed at my disposal during the course of this work, and to Dr. Francis W. Peabody for many helpful suggestions.

A STUDY OF THE DIAGNOSTIC AND PROGNOSTIC SIGNIFICANCE OF VENOUS PRESSURE OBSERVATIONS IN CARDIAC DISEASE*

ADMONT H. CLARK, M.D.
BALTIMORE

That high venous pressures occur in cases of cardiac decompensation has been observed by Gaertner,[1] Moritz[2] and von Tabora,[3] Frank and Reh,[4] Sewall,[5] Schott,[6] Hooker and Eyster,[7] and others. The routine measurement of venous pressure at frequent intervals as a means of following the stages of compensation or decompensation, however, has received little attention. The following investigation was undertaken with the purpose of ascertaining whether there is any diagnostic or prognostic significance in repeated venous pressure observations on cardiac cases.

METHOD

A number of instruments and methods have been devised by various workers to measure venous pressure. The technic and relative value of the methods used until 1914 have been summarized by Austin.[8] Of these, the direct method of Moritz and von Tabora of introducing a hollow needle directly into the vein, while perhaps giving a greater refinement of accuracy, would manifestly be open to serious objections in cases in which a large number of readings were made at frequent intervals. Probably the most useful clinical method was devised by Hooker[9] in 1914, and it is his instrument which has been used in this investigation. As shown in Figure 1, it consists essentially of a small glass cup (*B*) 2 cm. in diameter and 1 cm. deep, connected with a water manometer. The chamber is sealed to the skin over a suitable vein on the back of the hand by a rim of collodion. In drying, the collodion draws the skin slightly inward, so removing possible error due to superficial tissue tension. The manometer is connected

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* From the Medical Clinic of the Johns Hopkins Hospital.

1. Gaertner: München. med. Wchnschr., 1903, l, 2038.
2. Moritz and Von Tabora: Deutsch. Arch. f. klin. Med., 1910, xcviii, 475.
3. Von Tabora: München. med. Wchnschr., 1910, lvii, 1265.
4. Frank and Reh: Ztschr. f. exper. Path. u. Therap., 1912, x, 241; 1913, xiii, 37.
5. Sewall: Jour. Am. Med. Assn., 1906, xlvii, 1279.
6. Schott: Deutsch. Arch. f. klin. Med., 1912, cviii, 537.
7. Hooker and Eyster: Johns Hopkins Hosp. Bull., xix, 274.
8. Austin: Blood Pressure: Its Clinical Application, G. W. Norris, 1914, 129.
9. Hooker: Am. Jour. Physiol., 1914, xxxv, 73.

to the cup by a rubber tube, and by compressing the manometer bulb (*C*) the air pressure in the cup is increased. If the vein be observed by oblique illumination the point at which it definitely begins to collapse can be read in centimeters of water on the manometer scale. Hooker finds that "the most consistent results are obtained when the reading is made at the point where slight oscillations of pressure cause the vein shadow to come and go promptly just before the vessel is completely collapsed." This point has been used throughout this investigation. In all readings the same section of vein was constantly observed.

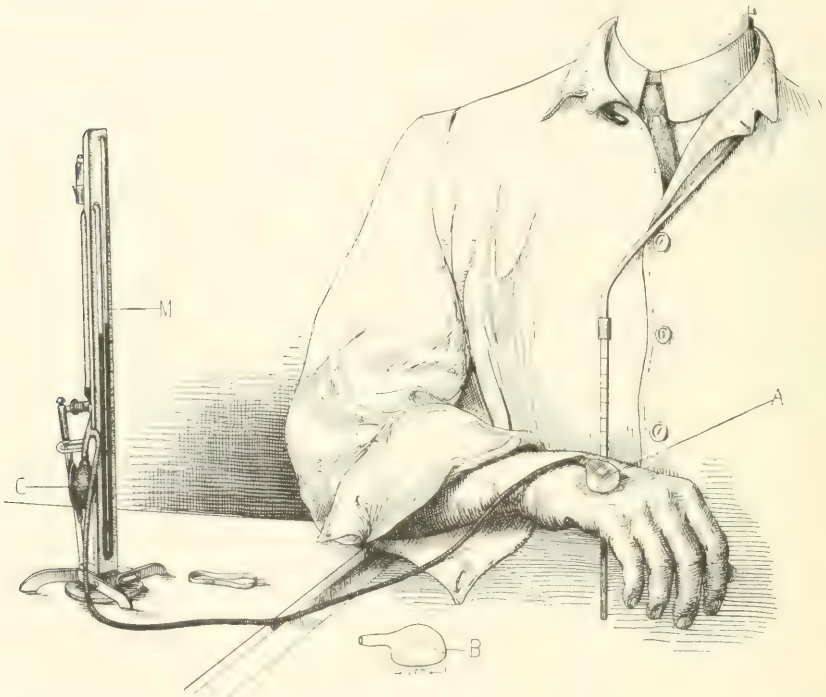

Fig. 1.—Hooker's venous pressure apparatus. A small glass chamber (*B*) measuring 1 by 2 cm. is held temporarily by a rubber band over a suitable vein on the back of the hand, as shown at (*A*). A rim of collodion is applied and in drying it seals the chamber to the skin. The rubber band is then removed and the chamber connected by a rubber tube to the water manometer (*M*). By pressing on the manometer bulb (*C*) the air pressure in the chamber is raised and a reading is made "at the point where slight oscillations of pressure cause the vein shadow to come and go promptly just before the vessel is completely collapsed." This pressure is recorded directly by the water manometer. The hand is held at the level of the mid point of the anterior-posterior diameter of the body at the costal angle.

As to the so-called "heart level," an arbitrary level in relation to the heart which must be chosen in order to give a constant point for making readings, the level originally defined by von Recklinghausen

and subsequently adopted by Hooker, has been used. This was chosen as the midpoint of the anterior-posterior diameter of the body at the costal angle. The hand, in each observation in this series, was placed on a pillow or other support at this level and was maintained quietly for at least three minutes to allow the hydrostatic factors to become adjusted and constant. As most patients with cardiac decompensation lie in bed with the body at an angle of about 45 degrees to the horizontal, this position was also adopted as the position in which to make the readings. In those cases with a venous pressure higher than the manometer could record, the hand was raised above the "heart level" until the venous pressure was within the range of the manometer. This hydrostatic column in centimeters was then added to the venous pressure.

It was further necessary to adopt the following three conditions: First, the vein must stand out sufficiently from the surrounding skin level to give a distinct shadow by oblique illumination. Second, the vein wall must be collapsible. Third, the patient must be lying quietly and undisturbed in bed.

Obviously not every cardiac case could be studied. Old patients with phlebosclerosis, patients with exceedingly edematous or fat hands, and patients with continuously small veins, had to be passed by. Nevertheless, excluding the rare cases of patients with hardened vessels there were few cases that did not show satisfactory veins on the back of the hand at some time during the day.

MATERIAL

Two hundred and seventy-six venous pressure observations have been made on fourteen cases at various stages of cardiac decompensation. The largest number of readings on a single case was seventy-three (Case 1), covering a period from November 5 to March 7. The lowest number was five (Case 14). In addition to the venous pressure, the systolic and diastolic arterial pressures by the auscultatory method, the pulse rate, the treatment, and the clinical condition of the patient were noted. The fluid intake and output for each twenty-four-hour period was recorded in the majority of cases. At each observation the venous pressure was taken before any of the other data in order to disturb the patient as little as possible. The highest venous pressure recorded was 50 cm. of water (Case 2) which exceeds any record found in the literature. This reading was made by two observers. A case was followed only so long as the venous pressure remained high or there was any doubt as to compensation. Five (Cases 2-6) of the fourteen patients died. In addition to these fourteen cases, eight cases were studied to determine the diurnal variation in venous pressure.

Before giving the results which were found, it would be well to state the factors which alter venous pressure, as observed by previous workers, especially in their relation to the present investigation. Barach and Marks[10] in a series of forty-eight normal cases noted a fall in venous pressure on passively changing from the erect to the horizontal position, and a rise of pressure in returning to the erect posture. Hooker,[11] Elpers,[12] and Schott[6] found that muscular exertion

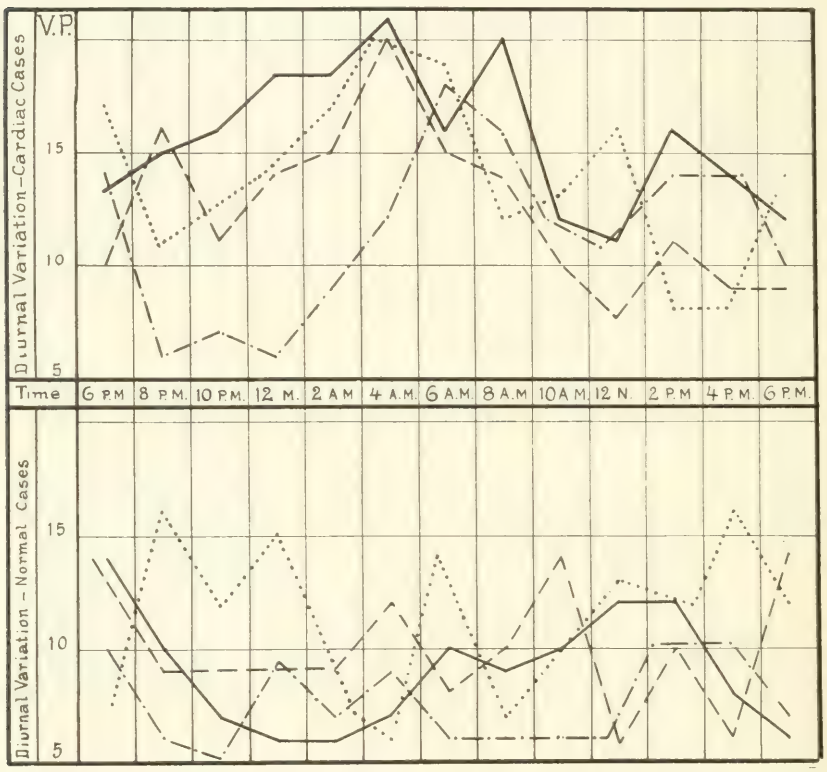

Fig. 2.—Curves showing the diurnal variation in four cardiac and four normal cases. The patients were all in bed under similar ward conditions. Note that in the cardiac cases the highest venous pressures occur at night, while in the normal cases there is an almost complete reversal of the picture, the lowest venous pressures tending to occur during the sleeping hours.

increases the venous pressure. The work of Elpers[12] would indicate that heat and cold increase the venous pressure, and finally, Hooker[9] has demonstrated a distinct diurnal variation in the venous pressure.

Obviously, for a patient lying quietly in bed under the conditions which were observed, the factors of temperature change, of muscular

10. Barach and Marks: The Archives Int. Med., 1913, xi, 485.
11. Hooker: Am. Jour. Physiol., 1911, xxviii, 235.
12. Elpers: Inaug. Diss., Kiel, 1911.

exertion, and of posture were practically constant. Under ideal experimental conditions these factors could hardly have been more carefully regulated. As to the complete diurnal variation under these conditions no data could be found, although Hooker[9] noted a distinct diurnal variation under normal conditions of health and a decided fluctuation in venous pressure during the day in three surgical cases confined to bed.

DIURNAL VARIATION

In view of these observations by Hooker, it was necessary, before it could be decided what constitutes a pathological venous pressure, to see what part the diurnal variation plays in the determinations made on patients confined to bed. To answer this question eight patients were selected on the medical wards. Only those cases which showed

TABLE 1.—Cardiac Cases

Case	Max. V. P.		Min. V. P.		Diurnal Variation Cm. H_2O
	Reading	Time	Reading	Time	
S.	21	4 a. m.	11	12 noon	10
T.	20	4 a. m.	8	12 noon	12
M.	20	4 a. m.	8	2– 4 p. m.	12
J.	18	6 a. m.	6	8–12 p. m.	12

TABLE 2.—Normal Cases

Case	Max. V. P.		Min. V. P.		Diurnal Variation
	Reading	Time	Reading	Time	
D.	14	6 p. m.	6	12 a. m.	8
..	..	.....	..	4 a. m.	
G.	14	6 p. m.	6	12–4 p. m.	8
..	..	.....	8	6 a. m.	
W.	10	6 p. m.	5	10 p. m.	10
L.	16	2 p.m.	6	4 a. m.	9
..	..	8 p. m.	..	.....	

excellent veins for reading were chosen. Four of these cases were at various stages of cardiac decompensation, and four had no cardiac complications. The observations were made at two-hourly intervals over a period of twenty-four hours. The glass cups were left attached during the entire period so that to make a reading it was only necessary to connect the manometer tube. All the patients were in the same ward environment under constant and similar conditions, and during

the twenty-four-hour period they remained in bed. There was a difference, however, between the cardiac and normal cases during the night, in that the normal patients lay flat in bed while the cardiac patients remained propped in a semi-erect posture. During the night it was possible to make the majority of readings without waking the patients, a small pocket electric light being used.

The eight cases are plotted in the curves shown in Figure 2. It is evident from these curves that there is a definite diurnal variation in venous pressure even under the constant conditions of the experiment. In comparing the curves of the normal and cardiac cases certain differences can be seen which are tabulated in Tables 1 and 2.

The first and striking fact is that in the cardiac cases the highest venous pressures occur in the early morning hours, while in the normal cases, as Hooker[9] found, the lowest venous pressures tend to occur at night. The second fact is that the diurnal variation averages 2.5 cm. more in the cardiac than in the normal cases. This greater change of pressure is interesting in connection with the observations of Schott,[6] that venous pressure can be altered by exercise in proportion to the degree of cardiac decompensation.

WHAT SHOULD BE CONSIDERED AS A PATHOLOGICAL VENOUS PRESSURE?

Since, therefore, a daily variation of from 8 to 12 cm. in venous pressure has been found in ward cases, the essential question is, what can be regarded as a pathological venous pressure? It is generally admitted that venous pressure observed at a fixed point and level is a fair indication of the feeding pressure of the heart. What, however, is the border-line between an efficient feeding pressure and a pressure due to an incompetent heart that cannot handle the blood with which it is supplied? With the factor of diurnal variation to be considered, an observation made one day might give the lowest and a reading the following day the highest pressure of a normal diurnal variation, and so one might be led to believe that there had been an abnormal increase in the venous pressure.

Various observers, depending on the method used, have given different values as to the upper limits of a normal pressure. Hooker,[9] by the method here used, gives 20 cm. as the upper limit of the normal diurnal variation under the varying conditions of daily life. To decide this point, the fourteen cases observed in this series have been classified according to the clinical divisions of "compensated or compensating" and "decompensated or decompensating." These divisions were evident from the clinical signs and symptoms. Of the six patients whose cases were classified as "decompensated," five died, and the sixth

(Case 1) recovered, though it was doubtful for three months whether he would live. At the time of writing none of the "compensated" cases have terminated fatally. All the patients have recovered or are on the way to recovery.

From Table 3 it will be seen that of 171 observations on six "decompensating" cases the average venous pressure in each case never went below 20 cm. while of 105 readings on "compensating" cases, the average of no case ever went above 20 cm. In this number of observations the factor of diurnal variation must be well equalized, for the readings were made at all hours of the day. The average venous pressure for all the "compensating" cases was about 14 cm. and of the "decompensating" cases 26 cm. It would seem, therefore, from these figures, that by this method, a venous pressure above 20 cm. is pathological, while any pressure below this may be regarded as within the limits of normal variation.

TABLE 3.—Compensated and Decompensated Cases

Case No.	Clinical Condition	No. Days Observed	Average Venous Pressure, Cm. Water
1	Decompensated	73	28.0 (first; 29 days 20.0 (last; 44 days
2	Decompensated. Died	33	29.7
3	Decompensated. Died	21	21.0
4	Decompensated. Died	8	23.2
5	Decompensated. Died	19	29.0
6	Decompensated. Died	17	29.3
		T'l 171	Avg. 26.0
7	Compensated	8	13.0
8	Compensated	30	10.7
9	Compensated	8	17.4
10	Compensated	25	14.0
11	Compensated	5	10.4
12	Compensating	9	15.2
13	Compensating	15	13.8
14	Compensated	5	19.0
		T'l 105	Avg. 14.3

It must be borne in mind, however, that a venous pressure below 20 cm., though indicating that a heart is handling the blood with which it is supplied, nevertheless, does not indicate how narrow the margin is between compensation and decompensation. Thus a heart barely compensating under perfect bodily quiet might give the same venous pressure as a well-compensated heart. Any unusual strain in the former case, however, would be sufficient to throw the heart back into decompensation, while in the latter case decompensation would not occur. It is on this consideration that Schott[6] based his venous pres-

sure test for the functional capacity of the heart. As shown above, however, the line of immediate danger seems to be at 20 cm. A pressure persisting above this gives a serious, and a pressure below a favorable, outlook.

CLASSIFICATION OF DATA

The deductions which follow are based on the observations stated under "Material" and are illustrated by the curves (Figs. 3-6) which demonstrate the most typical findings in the fourteen cases studied. The clinically interesting facts which they illustrate, follow:

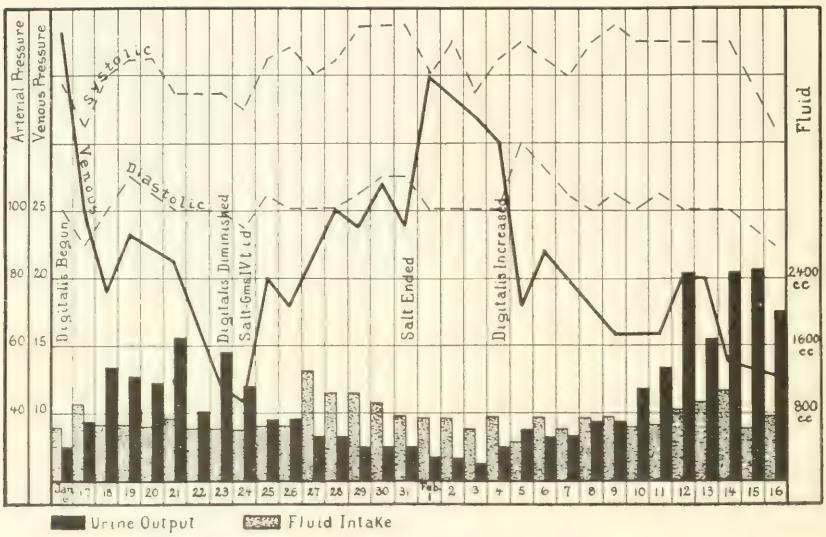

Fig. 3.—From the chart of Case 1, showing the relation of venous pressure, arterial pressure, fluid intake, and urine output. During the first fall in venous pressure the response in urine output is practically coincident, while after the second drop in pressure there is a delayed response of several days. Note also that the venous pressure gives an indication of the effect of therapy and compensation which are shown in no way by the arterial pressure.

Relation of Venous Pressure to Decompensation.—Of the seven cases which showed decompensation (Cases 1-8) all gave a direct relation between venous pressure as followed at frequent intervals and the degree of compensation as indicated by the clinical signs. A pressure continuously above 20 cm. almost invariably gave symptoms which called for treatment.

Rise in Venous Pressure Preceding a Break in Compensation.—In Case 1 (see Figs. 3 and 4), (November 12 to 18, November 23 to 30, December 3 to 11, January 12 to 18, January 26 to February 6), Case 2 (October 15 to 19, November 3 to 8, November 13 to 18, November 23 to 29), Case 3 (see Fig. 5), (November 25 to 27, December 7 to 9, December 12 to 20), Case 4 (see Fig. 6), Case 5 (October 16 to 26,

October 27 to November 9), and Case 6 (October 16 to November 7, November 10 to 19) a steady rise in venous pressure definitely preceded a break in compensation. When the venous pressure reached a high point the usual clinical signs of decompensation generally became apparent enough to call for treatment. If such treatment had been instituted before the venous pressure had risen to such a height the break in compensation might have been averted and the heart spared.

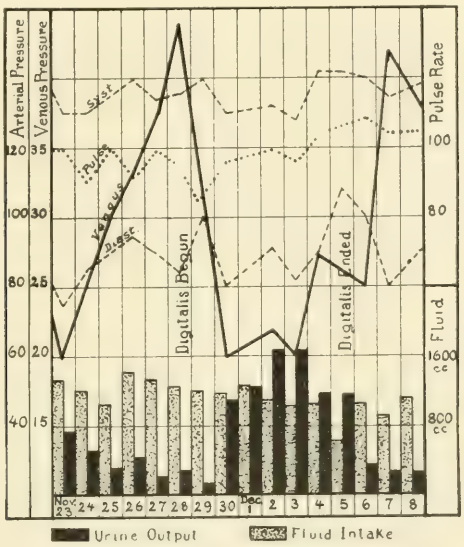

Fig. 4.—From the chart of Case 1, showing the relation of venous pressure, arterial pressure, and fluid intake and output. A rise in venous pressure and a fall in urine output preceded the clinical signs of decompensation which called for digitalis therapy. Note that neither the systolic nor the diastolic arterial pressures gave any indication of the degree of compensation. Only after the venous pressure had reached a high point did the clinical signs and symptoms become apparent enough to call for treatment.

Relation of Digitalis and Strophanthin to Venous Pressure.—All the patients in this series received digitalis or strophanthin either once or oftener during their stay in the hospital. Table 4 groups the results which were found.

TABLE 4.—Results of Use of Digitalis Series

Venous Pressure	Died	Compensating *	Decompensating
Definitely lowered by digitalis series.....	2,	10	1, 7
Not definitely lowered by digitalis series	3, 4 5, 7	8, 9, 11, 12, 13, 14	

* Average venous pressure, 14.

This table indicates that of the five cases which terminated fatally (See Figs. 5 and 6 as examples) only one showed lowering of the venous pressure as a result of cardiac tonics, while of the "compensated" cases also, only one showed a lowering of the venous pressure. In this latter case the variation was in the limits of normal. The lack of effect of digitalis or strophanthin on compensated cases would seem to be in accord with the experimental evidence of Capps and Mathews,[13] that the digitalis group does not materially alter the venous pressure in normal animals. The two decompensating cases in which the patients did not die gave a striking response to the digitalis group (See Figs. 3, 4, and 6).

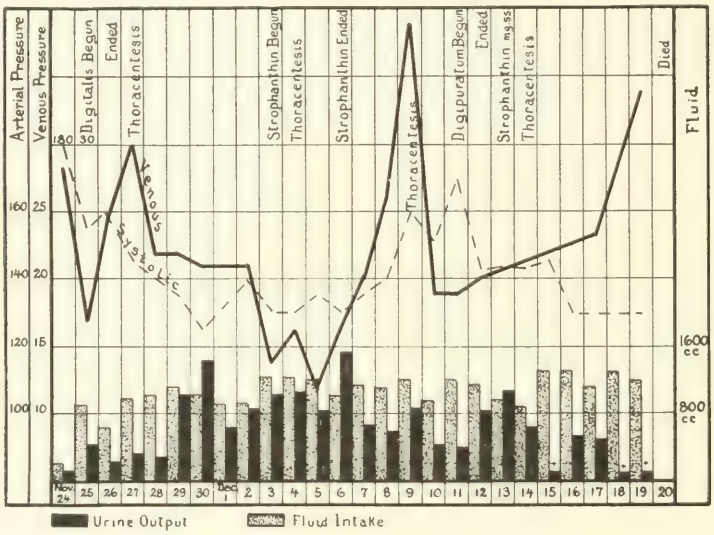

Fig. 5.—Case 3. Chart showing the relation of venous pressure, systolic arterial pressure, and fluid intake and output from admission to the hospital until death. The diastolic pressure could not be determined because of the extreme degree of aortic insufficiency. Note that from December 8 to 12 there was a coincident rise of venous and arterial pressures, but that before death the arterial pressure fell as the venous pressure rose.

If any conclusions can be drawn they would seem to be, first, that with the venous pressure continuously below 20 cm. the effect of digitalis cannot be followed by the venous pressure, and second, a venous pressure continuously above 20 cm., which is not lowered by digitalis, is an indication of grave cardiac involvement.

High Venous Pressure Before Death.—Of the five patients who died (2, 3, 4, 5, 6) all gave high venous pressure readings before death. Case 4 gave a reading of 38 cm. six hours before, while Case 3

13. Capps and Mathews: Jour. Am. Med. Assn., 1913, lxi, 388.

showed a pressure of 34 cm. fourteen hours before exitus. It was impossible to make readings on Cases 2 and 5 immediately before death, but so long as observations were possible, each showed, over a period of two weeks, average venous pressures of 37 cm. and 30 cm. respectively. Case 6, a typical cardiorenal, was continuously on the verge of severe decompensation. While under observation the patient's venous pressure averaged 29 cm. over a period of five weeks, and he died shortly after leaving the hospital. A rapid rise of venous pressure, however, in all the cases observed, did not invariably end unfavor-

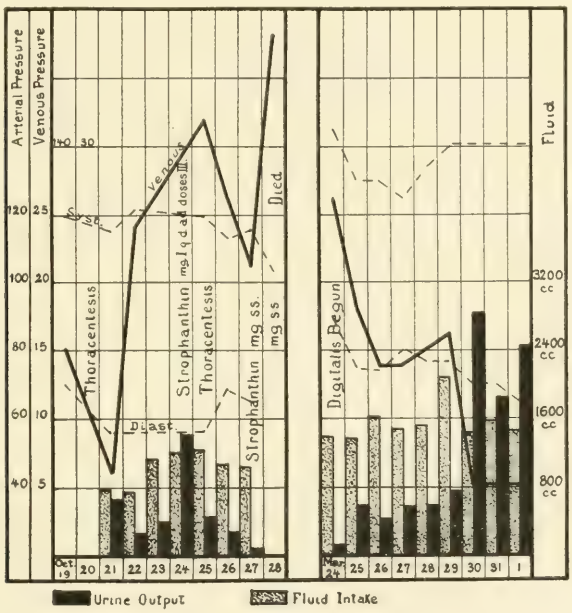

Fig. 6.—Case 4 (left), Case 7 (right). Charts of two cases of severe myocardial insufficiency, one ending in death, the other in recovery, showing the relation of venous pressure, arterial pressure, and fluid output and intake. Note that in the case which ended fatally the venous pressure steadily rose, the urine output diminished, and the systolic pressure fell with a lowering of the pulse pressure in spite of thoracentesis and strophanthin. In the case which ended in recovery the venous pressure fell rapidly, and the urine output and pulse pressure responded four days later to simple digitalis therapy.

ably, as can be seen by glancing at Figures 3 to 6. The proper therapy often lowered a high venous pressure. The interesting facts nevertheless remain, first, that so long as observations were possible no patient died with a low venous pressure, and second, that a rapid elevation of pressure to a high level, or a continuously high average venous pressure, was of serious prognostic significance.

Relation of Arterial to Venous Pressure.—The relation between arterial and venous pressure, as found in these cases, is interesting.

Plumier's[14] experiments on animals support the theoretical conclusion that venous and arterial pressure vary inversely. Elpers,[12] Schott,[6] and others, in human cases, have found that the height of venous pressure is not passively dependent on the height of arterial pressure. Isolated observations by me on several cases of extreme hypertension in which compensation was perfect, did not give high venous pressure readings. One case brought to the accident department with cerebral hemorrhage showed a systolic pressure of 300 mm., a diastolic of 180 mm. Hg, and a venous pressure of 20 cm. water. The relations which were noted between arterial and venous pressure are tabulated in Table 5.

TABLE 5.—Relations Between Arterial and Venous Pressures

	Venous Pressure Shows No Relation to	Venous Pressure Varies Directly with	Venous Pressure Varies Inversely to
Systolic arterial pressure	1 (at first), 2, 7, 10, 11	3 (at first), 5 (at first), 8, 9, 12	3 (before death), 5 (before death), 4, 6
Diastolic arterial pressure	1, 2, 4, 6, 9, 10, 11	7, 8, 12	
Pulse pressure	1, 2, 3, 4, 5, 6, 8, 9, 10, 11, 14	.....	7, 12
Pulse rate	1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 13	12	5

Thus, of 12 cases on which sufficient observations were made, 5 showed no relation between the systolic and the venous pressures; in 3, the venous and systolic pressures varied directly; 2 gave an inverse ratio between the pressures; while in 2 cases the venous and arterial pressure varied directly at first, and before death varied inversely. In 4 of the 5 cases which terminated fatally, the inverse ratio was noted, that is, the venous pressure rose and the systolic pressure fell before death.

Satisfactory diastolic pressure readings could be made in ten cases. Seven indicated no relation between diastolic pressure changes and venous pressure changes, while in three there was a fall in venous pressure with a fall in diastolic pressure. From the tables, however, it can be seen that this fall in diastolic pressure in Cases 8 and 12 was synchronous with a fall in systolic pressure but that in Cases 7 and 12 there was an increased pulse pressure due to the fall in diastolic pressure.

As to the relation of pulse pressure to venous pressure it was found that in eleven cases no relation was noticeable, while in two

14. Plumier: Arch. internat. de Physiol., 1909, viii, 1.

(See Fig. 6, Case 7 as example) there was a fall in venous pressure with an increased pulse pressure.

In this connection it is interesting to note the relation of the so-called "amplitude-frequency product" of v. Recklinghausen.[15] It was suggested by him that the product of the pulse pressure and pulse rate might be a criterion of the condition of the greater circulation. Fellner[16] thought he found a relation between this amplitude-frequency factor, and the venous pressure, in cardiac cases. Ten cases in this series could be compared as to this point. Of these, six, based on 173 observations, showed no definite relation, while four cases, based on 74 observations, showed only an occasional relation between the venous pressure and the amplitude-frequency product. In this series, therefore, neither the pulse pressure nor the product of the pulse pressure and the pulse rate seemed to be associated in any definite way with venous pressure.

To summarize the relation between the clinical observations of arterial and venous pressures in this series, it may be said that, except for a fall in systolic and a rise in venous pressure before death in four cases, no constant relation could be found between either the systolic, the diastolic, the pulse pressure, or the amplitude-frequency product on the one hand, and the venous pressure on the other; that in general, the venous pressure gave indications of the variations in compensation which were suggested in no way by the data obtainable from the arterial pressure.

Relation of Venous Pressure to Urine Output.—Clinically and experimentally it is well known that the function of a normal kidney depends largely on the circulation. With congestion and slowing of the blood stream the amount of fluid put out by the kidneys is diminished and with a relief of this congestion the kidney function returns to normal. Reference to Figures 3 to 6 shows a distinct relation between the venous pressure and the urinary output. In a total of 203 observations on ten cases in which the fluid intake and output were studied, this inverse ratio of venous pressure to urinary secretion was noted in seven (1, 2, 3, 4, 7, 10, 13) and was not found in two cases (8, 12). In six of these eight patients it was not apparent which was the first indication of the changed circulation, while in two instances (Figs. 3, and 6, Case 7) the drop in venous pressure preceded the polyuria by several days.

We would conclude, therefore, from this series that in the majority of cases the venous pressure and the amount of urine varied inversely and that the variations are coincident. This would add further evi-

15. Von Recklinghausen: Arch. f. exper. Path. u. Pharm., 1906, lvi, 1.
16. Fellner: Deutsch. Arch. f. klin. Med., 1907, lxxxviii, 1.

dence to show that consecutive changes in venous pressure are an indication of the condition of the circulation and heart.

Venous Pressure as Related to Intrathoracic Pressure.—Cases 2, 3 and 4 in this series had sufficient hydrothorax to make thoracentesis necessary. Eight pleural tappings were performed on these three cases. The venous pressure was lowered seven times with an improvement in the clinical condition of the patients. (See Figs. 5 and 6 as examples.) The final thoracentesis in Case 3 did not lower the venous pressure and the patient died. The intrathoracic pressure was measured twice with a water manometer in Case 3 and in each instance there was a change from a positive to a negative pressure during thoracentesis. This is in accord with the results of Hooker,[9] who found that increased intrathoracic pressure causes a rise in venous pressure which cannot be compensated. It also emphasizes the value of thoracentesis as a therapeutic measure in relieving an overstrained heart.

Effect of Venesection on Venous Pressure.—Table 6 illustrates this point in three cases observed. Venesection, therefore, seems to lower venous pressure, but the subsequent rise in pressure is rapid. The results of von Tabora agree with these observations.

TABLE 6.—Effect of Venesection on Venous Pressure

Patient	Condition	Amount of Blood Drawn c.c.	Fall in Venous Pressure cm.	Subsequent Effect on V. P.
S. H.	Emphysema, pulmonary congestion	500	28–9	Following day V. P. = 18
P. W.	Myocardial insufficiency. Pulmonary congestion	550	29–14	Following day V. P. = 28
L. M.	Normal, transfusion donor	540	18–9	Three hours later V. P. = 15

SUMMARY

1. The venous pressure has been followed at frequent intervals in fourteen cases of cardiac insufficiency at various stages of decompensation and compensation. Two hundred and seventy-six observations have been made. Hooker's modified method has been used, and the conditions which must be observed in making clinical venous pressure readings by this method have been given.

2. A diurnal variation in venous pressure of from 8 to 12 cm. of water was noted in four normal and four cardiac cases, all the patients

being confined to bed. This variation was based on two-hourly observations over a period of twenty-four hours. It was found that the highest venous pressures occurred during the sleeping hours in the cardiac cases, while in the normal cases, as previously observed by Hooker, the lowest pressures of diurnal variation occurred at night.

3. In the patients who died, either a rapid rise in venous pressure or a continuously high pressure above the 20 cm. level was observed before death.

4. A venous pressure continuously above 20 cm., which was not lowered by the digitalis series, was an indication of grave cardiac involvement.

5. A venous pressure continuously below 20 cm. gave no definite indication as to the effect of digitalis or strophanthin therapy.

6. A fall in systolic arterial pressure and a rise in venous pressure occurred in four cases before death. Otherwise no constant relation could be noted either between the systolic, the diastolic, the pulse pressure, or the amplitude-frequency product on the one hand, and the venous pressure on the other.

7. Venous pressure and urine output generally showed an inverse variation. The variation was usually coincident, but, if coincidence did not occur, the changes in venous pressure tended to precede the changes in kidney function.

8. The venous pressure was lowered in seven out of eight pleural tappings.

9. Venesection lowered the venous pressure in three cases observed, but the subsequent rise in pressure was rapid.

CONCLUSIONS

1. Venous pressure measurements, when made at frequent intervals, give a definite indication of the degree, and changes in the degree of cardiac decompensation.

2. A venous pressure of 20 cm., by this method, marks the danger line between compensation and decompensation.

3. A rise in venous pressure above 20 cm. precedes the clinical signs of decompensation.

4. Above the 20 cm. level a rising venous pressure has an unfavorable, and a fall in venous pressure a favorable prognostic significance.

5. Death from cardiac decompensation is preceded by a continuously high venous pressure, or a rapid rise.

6. Venous pressure observations at short intervals give information as to the degree of cardiac decompensation which is not obtainable by observation of the arterial pressure.

7. Frequent venous pressure measurements not only have a valuable diagnostic and prognostic significance, but also furnish an important indication for, and check on therapeutic measures.

Grateful acknowledgment is made to Dr. D. R. Hooker, who supplied the instrument used, and to the clinical staff of the hospital and others who kindly cooperated in this investigation.

PROTOCOLS

Case 1.—J. S., aged 48, male, colored. Admitted Oct. 28, 1914. Clinical diagnosis: Aortic insufficiency and myocardial insufficiency. Eleven months before admission began having shortness of breath and swelling of the abdomen. Was improved by three weeks' stay in the hospital during April, 1914. Symptoms returned a few weeks before last admission. Admitted with dyspnea, orthopnea, edema of the legs, ascites, moderate hydrothorax, a large tender liver, greatly enlarged area of cardiac dulness and signs of aortic and mitral regurgitation. Under digitalis, purgation, diuretics, and limited fluids the patient wavered between compensation and decompensation for about six weeks, then went on to recovery. The venous pressure from November 5 to February 7 varied from 44 to 10 cm. The average venous pressure for the first twenty-nine days was 28 cm., and for the last forty-four days 20 cm.

Case 2.—33,167. F. J., aged 49, female, colored. Admitted Oct. 12, 1914. Clinical diagnosis: Aortic insufficiency and myocardial insufficiency; syphilis. Three weeks before admission the patient began having shortness of breath, orthopnea, cough and edema of the ankles and feet. Admitted to the hospital with dyspnea, orthopnea, tachycardia, and enlarged area of cardiac dulness, the signs of aortic insufficiency and fluid at the bases of both lungs. Under limited fluids, purgation, thoracentesis, diuretics, digitalis and strophanthin the patient wavered between compensation and decompensation for five weeks, but finally no longer responded to treatment and died December 15. Necropsy confirmed the clinical diagnosis. The venous pressure from October 15 to December 4, when it could no longer be observed because of the edema of the hands, varied from 9 to 50 cm., with an average pressure of 29.7.

Case 3.—33,356. S. S., aged 45, female, white. Admitted Nov. 24, 1914. Clinical diagnosis: Aortic insufficiency and myocardial insufficiency; syphilis. Five months before admission patient began having paroxysmal attacks of dyspnea, orthopnea, severe epigastric pain and edema of the ankles. On admission was dyspneic, orthopneic, with ascites, anasarca, hydrothorax and showed a large area of cardiac dulness and the signs of aortic and relative mitral insufficiency. In spite of repeated thoracentesis, limited fluids, purgation, diuretics, digitalis and strophanthin the patient did not improve and died about a month after admission. Necropsy confirmed the clinical diagnosis. The venous pressure observed from admission until death varied from 12 to 39 cm., with an average pressure of 21 cm.

Case 4.—33,172. E. W., aged 20, female, white. Admitted Oct. 13, 1914. Clinical diagnosis: Acute and chronic endocarditis; mitral stenosis and insufficiency; myocardial insufficiency. At 14 years patient began having palpitation and shortness of breath. Ten months before admission became pregnant and as pregnancy advanced, dyspnea and palpitation became progressively worse. Following delivery on October 6 the patient became no better and in spite of repeated thoracentesis, limited fluids, purgation, diuretics, strophanthin and digitalis, the patient was not improved and died fifteen days after admission. Necropsy confirmed the clinical diagnosis. Venous pressure from October 19 until death varied from 15 to 38 cm. with one reading of 6 cm. immediately after thoracentesis. The highest venous pressure of 38 cm. occurred six hours before death.

Case 5.—33,181. P. V., aged 52, white, male, admitted Oct. 15, 1914. Clinical diagnosis: Aortic and mitral stenosis and insufficiency; myocardial insufficiency. About a year before admission began noticing shortness of breath and swelling of the ankles. Was compelled to stop work. Was improved by five weeks' stay in the hospital in February, 1914, but soon relapsed into his previous condition. Admitted with dyspnea, orthopnea, anasarca, ascites, a large and tender liver and spleen, and the signs of aortic and mitral stenosis and insufficiency, and myocardial insufficiency. Was not improved by limited fluids, Karell diet, purgation, diuretics, digitalis, or venesection, and died four weeks after admission. Necropsy confirmed the clinical diagnosis. Venous pressure from October 16 to November 10 varied from 16 to 39 cm., with an average pressure of 29 cm.

Case 6.—33,104. W. H., aged 52, white, male. Admitted Sept. 30, 1914. Clinical diagnosis: Arteriosclerosis; chronic nephritis; hypertension; myocardial insufficiency. For five years had slowly increasing dyspnea, weakness, edema of the ankles and swelling of the abdomen. For almost a year had been able to do no work. Admitted to the hospital with dyspnea, orthopnea, polypnea, ascites, general anasarca, a much enlarged area of cardiac dulness, a blood pressure of 220, right-sided hydrothorax and albuminuria. Phenolsulphonephthalein excretion 15 per cent. in two hours. Showed no improvement under limited fluids, purgation, diuretics and digitalis. Remained in hospital for seven weeks and died shortly after returning home. Venous pressure from October 16 to November 23 varied from 20 to 41 cm. with an average pressure of 29 cm.

Case 7.—33,922. P. W. R., aged 66, white, male. Admitted March 23, 1914. Clinical diagnosis: Myocardial insufficiency; auricular fibrillation. Dyspnea and palpitation on exertion for the past ten years. Symptoms became severe two years ago with swelling of the legs and abdomen. Was relieved by treatment but has had frequent recurrences. Admitted to the hospital with dyspnea, orthopnea, considerable edema and ascites and enlarged area of cardiac dulness, auricular fibrillation, and an enlarged tender liver. Under limited fluids, purgation and digitalis he improved rapidly and was discharged two weeks after admission. Venous pressure on admission was 26 cm., but fell in a week to 5 cm. The fall in venous pressure preceded a great increase in urine output which came eight days after admission.

Case 8.—33,350. A. T., aged 44, colored, male. Admitted Oct. 4, 1914. Clinical diagnosis: Myocarditis; myocardial insufficieny. Cardiac symptoms began one month before admission with dyspnea, cough and edema. Examination showed an enlarged area of cardiac dulness with the signs of myocardial insufficiency. Was treated for four weeks and discharged considerably improved. Returned to work but in three weeks another break in compensation occurred. The patient was readmitted to the hospital with the signs of decompensation. Under digitalis, purgation, limited fluids and diuretics the patient's condition was improved and he was discharged after eight weeks in the hospital. The venous pressure on first admission varied from 10 to 24 cm. and during the second admission (November 22-January 19) varied from 18 to 5 cm., with an average pressure of 10.7 cm.

Case 9.—33,166. C. A., aged 34, colored, male. Admitted Oct. 12, 1914. Clinical diagnosis: Aortic, mitral and myocardial insufficiency. History of rheumatism at 16 years. Cardiac symptoms began in August, 1914. Was improved by two weeks' treatment in the hospital. Returned to work and had his second break in compensation. Was readmitted to the hospital with dyspnea, orthopnea, tachycardia, cough, rusty sputum, râles at both lung bases, an increased area of cardiac dulness and the signs of mitral and aortic insufficiency. Improved somewhat by Karell diet, purgation, digitalis and diuretics and was discharged seventeen days after admission. The venous pressure varied from 28 to 12 cm. between October 15 and 29, with an average pressure of 17.4 cm.

Case 10.—33,275. C. D., aged 49, white, female. Admitted Nov. 5, 1914. Clinical diagnosis: Arteriosclerosis, hypertension, myocardial insufficiency, chronic nephritis. Housewife with good general health until one year ago when she began having dyspnea, palpitation, orthopnea, edema of the legs and nocturnal polyuria, which became steadily worse. On admission, the patient showed albuminuria, a blood pressure of about 200/140, slight edema of the feet, a moderately dilated heart with signs of myocardial insufficiency, and occasional attacks of paroxysmal dyspnea. Under digitalis, purgation, diuretics and rest, the patient improved somewhat and was discharged seven weeks later. Venous pressure observed from November 12 to December 21 varied from 20 to 9 cm., with an average pressure of 14 cm.

Case 11.—33,154. D. M., aged 46, white, male. Admitted Oct. 10, 1914. Clinical diagnosis: Mitral insufficiency; myocardial insufficiency; arteriosclerosis; tuberculosis (?). Alcoholic history. Thirteen months before admission began having epigastric pain, shortness of breath and palpitation. Admitted with a slightly enlarged liver, mitral disease and doubtful myocardial insufficiency. Wassermann positive. Suspicious signs of pulmonary tuberculosis. Digitalis and limited fluids. Discharged eight weeks after admission in improved condition. Venous pressure from October 14 to 22 varied from 12 to 8 cm., with an average pressure of 10.4 cm.

Case 12.—33,531. J. F., aged 42, colored, male. Admitted Jan. 3, 1915. Clinical diagnosis: Syphilis of aorta; aortic insufficiency. Two weeks before admission the patient began having cough, shortness of breath and palpitation. On admission the patient was dyspneic and showed the signs of aortic insufficiency and moderate dilatation. Wassermann positive. Digitalis, purgation and limited fluids. Discharged in three weeks with the heart well compensating. Venous pressure from January 6 to 24 varied from 22 to 10 cm., with an average pressure of 15.2 cm.

Case 13.—T. T., aged 42, colored, male. Admitted Jan. 19, 1915. Clinical diagnosis: Myocardial insufficiency and aortic insufficiency. Fourth admission to the hospital since March, 1913, for recurrent breaks in cardiac compensation. Admitted with dyspnea, orthopnea, edema, moderate ascites and an enlarged heart with the signs of aortic insufficiency. Under restricted fluids, purgation and digitalis his condition improved gradually. Venous pressure, observed from January 26 to February 16, varied from 20 to 10 cm., with an average pressure of 13.8 cm.

Case 14.—33,420. W. H. G., aged 51, colored, male. Admitted Dec. 7, 1914. Clinical diagnosis: Myocardial insufficiency; syphilis of aorta. Patient had periodic attacks of dyspnea and palpitation for six years before admission but no edema. Last attack began five weeks before admission and was accompanied by edema of the ankles and swelling of the abdomen. Symptoms became steadily worse and on admission the patient was dyspneic, orthopneic, showed a dilated area of cardiac dulness, auricular fibrillation, a large tender liver, shifting dulness in the flanks, and edema of the ankles. Wassermann positive. Karell diet, limited fluids, purgation, digitalis and strophanthin. Discharged in five weeks with compensation well established. Venous pressure from December 11 to January 10 varied from 22 to 16 cm., with an average pressure of 19 cm.

STUDIES ON THE RELATIONS OF THE HYPERSUSCEPTIBILITY AND INSUSCEPTIBILITY INDUCED IN GUINEA-PIGS BY THE INSTILLATION OF HORSE SERUM INTO THE NOSE *

HENRY SEWALL, M.D., AND CUTHBERT POWELL, M.D.
DENVER

In the course of an investigation detailed by one of us a year ago,[1] it was found that when a normal guinea-pig was treated by a series of instillations of horse serum through the nose the results of the treatment, as tested by subsequent intravenous injections of horse serum, were always positive but in two opposite directions. In one group of animals the first intravenous injection of serum caused speedy "anaphylactic" death; that is, the individuals had been highly sensitized to horse serum by the instillations. In the other group of animals the first intravenous injection produced either no reaction or a variable degree of shock, and a second injection after an interval of three weeks was likewise withstood.

We have sought, in the present research, to determine the experimental conditions under which might be produced at will either increased specific sensitiveness or magnified resistance to the operation of the antigen.

It seemed as if the modification of vital irritability into hyperinsensitiveness or hyposensitiveness might depend on the time interval elapsing between successive serum treatments; therefore the first task we set ourselves was to determine the effect of varying this interval as measured by the reaction of the guinea-pig to subsequent intravenous injection.

The antigen used by us was uniformly the "normal horse serum" prepared at the Cutter Laboratory, Berkeley, California.

There are strong *a priori* reasons why the mucous membrane of the nose deserves especial consideration as an avenue through which the immunological processes of the organism may be set in motion. It seems to us to be the organ, *par excellence*, through which the *natural* reactions between antigens and the living tissues are mediated. An animal could conceivably die of old age without ever experiencing an infective scratch of the skin, but every breath of air inhaled contains its suspension of organic matter liable to settle on the nasal channel

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1. Sewall: THE ARCHIVES INT. MED., 1914, xiii, 856.

and there set up profound reactions. Moreover, it seems probable that the dosage of serum absorbed through the application of a few drops to the mucous membrane of the nose must belong to the same order of values as the noxa of pathogenic germs which play so large a part in the infective disorders of man. We must suspect that the biological reactions in the one case are closely paralleled by those of the other.

A single instillation of foreign serum does not, as tested by subsequent "toxic" injections of the same antigen, have any effect that we have been able to discover. But when, at most, five successive instillations have been made, even at intervals of twenty-four days, the results are positive enough. We may say then that each stimulus conveyed in the serum treatment is subminimal in intensity but that the tissues have the ability to summate the effects of individual stimuli and thus be brought to manifest definite reactions, much as the spinal cord is able to integrate the effects of discrete subminimal electric shocks and set forth a reflex action. It seems reasonable to suppose that biologic problems are simplified in proportion to the diminution in intensity and number of reactions entering into them.

LITERATURE

Observations are not wanting on the effects of the introduction of antigen by the natural channels of the body.[2] Sensitization to proteins has been accomplished by their application to various mucous membranes, as that of the eye, the nose, the stomach, the rectum. There is, however, a note of uncertainty in the claims of those who have used these avenues of sensitization; especially is this so in the description of the effects of oral administration of antigens, their sensitizing properties being often annulled by the action of the digestive juices.

Only among those who, like Dzerjgowsky[3] and Blumenau,[4] have practiced immunization through the nose do we find enthusiastic advocates of the choice of what may be called "physiological" channels of sensitization.

Trenchant evidence that absorption of an antigen through the mucous membrane of the nose determines a biological response different in degree if not in kind from that of the same antigen injected beneath the skin is presented by the experiments of various observers

2. For literature see Kolle and Wassermann: Handbuch. d. path. Mikroorg., 1913, ii. 1, 134, 136. Also Vaughan's "Protein Split Products."

3. Dzerjgowsky: Ztschr. f. Immunitätsforsch. (abstr.) 1910, iii, 602.

4. Blumenau: Ztschr. f. Immunitätsforsch., Ref. Bd., 1911, pp. 196, 353, 860; Jahrb. f. Kinderh., 1911, lxxiv, 141.

5. Anderson: Bull. 30 Hyg. Lab., 1906.

with diphtheria toxin. Anderson[5] tried in vain to immunize guinea-pigs to diphtheria toxin by subcutaneous injection. He writes:

> Doses considerably less than the M. L. D. and not sufficient to cause a perceptible reaction were given at intervals of about seven days. Before an amount equal to an M. L. D. had been given the animals in each case died. These results are in exact accord with the work of Behring and Kitashima, who found that guinea-pigs, when given daily injections of diphtheria toxin and when the total amount injected was only 1/400 of an M. L. D. after a time died with the postmortem lesions of poisoning from diphtheria toxin. Instead of being able to produce an immunity to the toxin a hypersusceptibility was produced.

Dzerjgowsky[3] found it possible to achieve in man and animals a mild degree of active immunity by carefully conducted subcutaneous injections of diphtheria toxin carried on over many months; nevertheless he became the enthusiastic pioneer in the field of absorption by the mucous membranes of the respiratory passages. In his work diphtheria toxin was inhaled in sprays or administered on cotton plugs soaked with *undiluted toxin* placed in the nostrils. Blumenau[4] repeated these observations in various ways on a large number of children. In one series of experiments, cotton plugs heavily soaked with undiluted toxin were placed for an hour daily in alternate nostrils for a period of one or two weeks. Like his predecessor, he found the procedure to cause no subjective symptoms but to be followed by the appearance of a small amount of antitoxin in the blood.

Scheweleff[6] carried out similar experiments on dogs and found he could produce active immunization against diphtheria.

The incubation period following an inoculation and preceding the full development of hypersensitiveness probably depends on the dosage of antigen. Though Rosenau and Anderson[7] could find no difference in the incubation period with varying dosage, more recent opinions are in accord that with very small doses of antigen, approaching the minimal capable of producing effect, the latent period of sensitization is greatly prolonged.

While a guinea-pig injected with a moderate amount of foreign protein reaches, according to Besredka,[8] a fairly complete degree of sensitization in twelve days, Weil[9] found that when 0.01 c.c. of horse serum was given subcutaneously full sensitization was not manifested by the guinea-pig until the lapse of sixteen days. Especially useful to our present purpose is the research of Doerr and Russ.[10] These authors treated guinea-pigs with subcutaneous injections of ox-serum.

6. Scheweleff: Ztschr. f. Immunitätsforsch., Ref. Bd., 1910, x, 1085.
7. Rosenau and Anderson: Jour. Med. Research, 1908, xx, 37.
8. Besredka and Steinhardt: Ann. de l'Inst. Pasteur, 1907, xxi, 384.
9. Weil: Jour. Med. Research, 1913-14, xxix, 233.
10. Doerr and Russ: Ztschr. f. Immunitätsforsch., 1909, ii, 109.

They found that animals treated with sensitizing doses of from 0.01 c.c. to 0.001 c.c. invariably succumbed to the intravenous injection of 0.2 c.c. of serum after eight days. With sensitizing doses of 0.001 to 0.00001 c.c. the toxic injection was not fatal until the lapse of nineteen to twenty-five days.

The subcutaneous injection of 0.000,001 c.c. (1/1,000,000 c.c.) did not cause hypersensitiveness at all. They call attention to the enormous difference between the minimal sensitizing and the minimal (fatal) toxic dose, the ratio being 1 to 1,000.

Rosenau and Anderson[11] found that guinea-pigs could be occasionally sensitized by a subcutaneous injection of 0.000,001 c.c. horse serum. White and Avery[12] say that guinea-pigs when sensitized by the intraperitoneal injection of 0.000,0001 gm. of edestin react fatally to the intravenous injection of 0.05 gm. of edestin after twenty-one days.

Since in our work only negative results were obtained after a single nasal instillation of serum while profound effects could follow two successive instillations, we may possibly be justified in assuming that the amount of antigen absorbed at each instillation was approximately 0.000,001 gm.

Authors agree fairly well that free antibodies first appear in the blood of an inoculated animal after a latent period of eight to ten days. Wells[13] summarizes the evidence as follows:

> In active sensitization it [anaphylactin — the antibody] appears in the blood in appreciable quantities in about eight days after the sensitizing injection, increases to a maximum between the fifteenth and thirtieth days, and then very slowly decreases.

Dale[14] found that in the uteri of young guinea-pigs sensitized to 0.1 c.c. horse serum, tested in vitro as to their sensibility to horse serum, up to and including the sixth day (after the sensitizing dose) there was no sign of response to the highest dose employed. On the eighth day one horn of the uterus gave a trifling and doubtful response. On the tenth day the reaction was much more obvious and on the twelfth and fourteenth days the response was maximal.

Besredka and Steinhardt[8] investigated on guinea-pigs the effect of massive, 2 to 5 c.c., intraperitoneal injections of horse serum given at various periods after the subcutaneous sensitizing dose. They found that when the second injection was given within twenty-four hours of the first it had no effect; that is, sensitization proceeded as if from the first dose only. When the second injection followed the

11. Rosenau and Anderson: Bull. 29, Hyg. Lab., 1906.
12. White and Avery: Jour. Infect. Dis., 1913, xiii, 103.
13. Wells: Chemical Pathology, 1914, p. 185.
14. Dale: Jour. Pharm. and Exper. Therap., 1913, iv, 167.

first later than one day and within any period up to twelve days the animal, while manifesting no reaction, became "vaccinated" against the serum and refractory to a subsequent inoculation. A succession of massive intraperitoneal injections given, say, at eight-day intervals, likewise made the animals refractory. They consider this insensitive condition to be one of antianaphylaxis in which the animal is desensitized and returned to the normal state. They say that this refractory state may be induced, in a guinea-pig which has received a sensitizing injection, in two ways: either by a second large intracerebral injection of the antigen (¼ c.c.) given within ten days of the first or by a small (1/40 c.c.) dose given after complete sensitization. The "immunity" conferred by vaccinating a sensitized guinea-pig lasts three months and probably longer.

Besredka's statement that the "desensitizing" injection restores the animal to the normal state cannot hold for cases, such as are described in the present paper, in which a series of separate toxic injections are withstood.

R. Weil[15] found no difference between animals receiving single or multiple sensitizing injections except that in the latter the blood contains more immune bodies. He injected 3 c.c. foreign serum subcutaneously in guinea-pigs on four successive days. Toxic intravenous injections given on the eighth and eleventh days produced no symptoms. Given from the thirteenth to fifteenth day they killed.

Rosenau and Anderson,[16] after an elaborate investigation, concluded that guinea-pigs injected subcutaneously with a succession of large doses of horse serum within the period of incubation were partially immunized, while by similar treatment with small doses they were only sensitized. These authors gave ten subcutaneous injections of 2 c.c. of normal horse serum covering a period of seventeen days. In seventeen days thereafter an intraperitoneal injection of 6 c.c. serum produced only mild symptoms. Five subcutaneous injections of 0.001 c.c. of serum given within a period of eight days led, in most cases, to fatal shock from a toxic injection after twenty-three days.

METHOD AND SCOPE OF WORK

The introduction of serum into the nose of a guinea-pig is an extremely simple procedure. As described in a previous article,[1] our method was to clasp the animal's head with the left hand and to hold it gently supine on a table. The serum was dropped from an all-glass hypodermic syringe having a blunted needle. We cannot

15. Weil: Jour. Med. Research, 1913, xxviii, 243.
16. Rosenau and Anderson: Bull. 45, Hyg. Lab., 1908.

pronounce on the minimum quantity of serum sufficient to produce constitutional effects.

The serum was used in full strength and as nearly as possible 3 minims (approximately 0.19 c.c.) was used at each instillation. The droplets of serum were allowed to fall slowly on one or the other nostril, about five minutes being consumed in each instillation.

Usually but one nostril was utilized at one sitting and the other at the next. In this way the animal was allowed a clear breathing space through the experiment. On a few occasions the dose of serum was distributed into both nostrils, thus probably doubling the absorbing surface put in play; this made no noticeable physiological difference. The guinea-pig treated in this way manifests a wide variety of behavior. In our previous work[1] it was stated that there appeared to be a sharp distinction between the reactions exhibited by the wholly normal and the more or less sensitized pig. The characteristic demeanor of the former animal is one of quiescence; the droplets of serum are sucked in as soon as they fall on the naris. In the second there is a good deal of nasal irritability; the serum excites sniffing and expulsive movements and more or less violent struggles of the whole body.

Several hundreds of additional observations, however, have shown that often wholly normal animals would manifest considerable irritatation with the first application of serum while those that had been treated remained quiescent during subsequent instillations. A very common reaction, occurring at both the first and the subsequent treatments, was the secretion of saliva which would well out from the mouth within a few seconds of the instillation of three or four droplets of serum. This excessive secretion of saliva is probably habitual, but its accumulation is prevented by swallowing movements.

The foregoing description applies to reactions from animals treated regularly at intervals less than twenty-four days. More characteristic symptoms are apt to be manifested by pigs which, after daily treatment for three or four times, are allowed to rest for sixteen days before the next instillation, or by pigs at about the fourth treatment in a series with twenty-four days' interval.

More especially in such cases characteristic symptoms of sensitization appear. Two or three minutes after beginning the instillation, more or less active movements of intestinal peristalsis are seen. If the pig is a noticeably pregnant female, twitching of the lower abdominal wall is obvious, and if pregnancy is far advanced, within a few days of term, there are very lively fetal movements which seem, when felt by the hand, to be active fetal contractions. We have not been able to assure ourselves that these movements are not imparted

to the fetus by vigorous uterine contractions. The distinction is one of theoretical importance and the question deserves special investigation. During the fetal turmoil the mother remains passive. Succeeding the onset of intestinal peristalsis the respiratory movements are apt to become deeper, and in about one-fifth or more of the cases clicking râles are felt in the throat, then râles are heard in the nose and extend to the bronchial tubes, the animal coughs, becomes dyspneic and is involved in the throes of a bronchial asthma which is almost identical in its symptoms with an asthmatic attack in the human subject. The respiratory urgency gradually passes off in about half an hour, but the animal may still be disturbed after several hours. We can state definitely that the more marked are these signs of *quasi* local sensitization the more likely is the animal to succumb to the toxic injection.

In pigs which have been highly sensitized by a single subcutaneous injection, serum dropped into the nose is apt to cause, after a certain number of treatments, immediate frantic signs of distress to be succeeded in a few minutes by an urgent asthma.

It might be objected that the introduction of an antigen by the nose gives no assurance of the site of its absorption. Some of it may reach the trachea and some is certainly swallowed. We can only say that nothing could be more positive and constant than the systemic reactions that follow such procedures as we describe, whereas the results of those who have sought to sensitize by the stomach seem to be very variable.

In our work already quoted[1] it was proved that the olfactory apparatus per se has no necessary relations to the sensitizing effects of serum introduced into the nose. Also it was found that specific differences marked the reactions resulting from the nasal instillation of horse serum and inhalation of the serum in a spray. Rosenau and Anderson[16] describe a mild degree of sensitization resulting from the instillation of serum into the conjunctival sacs of guinea-pigs. It is obvious, of course, that the antigen might have produced its effect through the nasal mucous membrane after traversing the tear duct.

It is futile to speculate as to the mode of action of the mechanism of absorption. The mucous membrane of the nose presents an immense colloidal surface, which we may imagine to adsorb the foreign protein and give it off little by little to the circulation.

The experimental evidence is positive that specific reactions occur, in certain animals, within about one to three minutes of the time of beginning a treatment by instillation.

Our guinea-pigs were divided into various groups, each of which was utilized in the solution of a definite problem. The first nasal instillation was by the right nostril, the second, after a predetermined

interval of from one to twenty-four days, was by the left nostril, and so on in alternation until the desired number of instillations had been given. Sixteen days after the last instillation there was given an intravenous injection of 6 minims, approximately 0.4 c.c., of horse serum. The absence of a reaction might indicate either a refractory condition of the animal or that the instillation had had no demonstrable effect. Therefore, in case of survival, a second intravenous injection of the same amount of serum was given twenty-four days after the first. When the animal still survived, subsequent intravenous injections were given at irregular, longer intervals up to more than one hundred days.

The injections were made under such light ether anesthesia that when no shock was produced, the animal sat up at once and behaved about as normal as soon as released from the holder.

Friedberger and Mita[17] found that when intravenous injections were carried out with sufficient slowness, through ten minutes or longer, a large amount of serum might be tolerated by sensitized animals. In our later work the time occupied in injecting the serum was regulated. Our usual injection period for six minims of serum was thirty seconds. This period was frequently doubled or trebled, without, in most cases, apparent influence on the result.

The operative technic was carried out wholly by one of us (C. P.) who found that the preferable intravenous route was in a small vein found constantly just under the skin of the upper foreleg. When the guinea-pig is stretched on its back an incision made along the uppermost border of the leg lays this vein bare. The thin sheath is cleared from its upper surface, but the vein is allowed to remain in its bed. The leg of the animal being free, the operator clasps it with the left hand, retracting the lips of the wound with finger and thumb. A fine, sharp hypodermic needle attached to a glass syringe is inserted, the vein being held only by its natural attachments, and the serum injected toward the heart. The minute puncture is closed by passing the first stitch under its site.

The guinea-pig which has been highly sensitized by subcutaneous injection, on receiving an intravenous dose of the antigen quickly shows respiratory distress, deep forced breathing movements, but speedily, in addition, general convulsions usually involve the whole musculature of the body, the spasms having no sensible connection with the respiratory function. In animals sensitized through the nose the general anaphylactic convulsion is much less pronounced. The distress limits itself more nearly to the respiratory apparatus. A pig

17. Friedberger and Mita: Deutsch. med. Wchnschr., 1912, xxxviii, 204.

may throw a forward somersault in an obvious effort to get the air out of its lungs. On several occasions we are confident that we have saved the lives of badly shocked pigs by compressing their chests with the hand, the air being expelled with a moist, whistling sound. This procedure is in most cases entirely ineffective. The experience suggests, however, that in certain cases of anaphylactic shock—at least in the nose-treated animals—death is due not to a toxemia but simply to mechanical interference with lung ventilation. The fortunate outcome of the application of suitable artificial respiration suggests similar treatment in certain human accidents.

We have never seen a delayed toxic reaction in our nasally treated guinea-pigs. In other cases which we have seen in which death was deferred for an hour or more after the toxic injection the lungs did not present the anaphylactic distention. We wish to imply that in the nasally treated pigs anaphylactic fatality seemed to depend on mechanical obstruction to the air exchange without evidence of toxic changes in the vital centers. Gay and Southard[18] have presented interesting observations in this connection.

ORIGINAL EXPERIMENTS

The results of our first set of observations are summarized in Table 1. As our object was primarily to determine the specific influence of the interval elapsing between successive instillations our animals were divided into groups on this basis.

In Column 4 the intervals in days between successive instillations are recorded; with a few noted exceptions the two nostrils were treated in alternation. Each animal is identified by a number in Column 1. The weight of each animal in grams is recorded at the beginning and again at the end of its experimental career. The results following the intravenous or "toxic" injections of serum are recorded in Columns 6 to 10. When an animal survived such an injection (L) the degree of reaction manifested is represented by the signs + (one or more) and 0; 0 signifies no perceptible effect—the animal sits up at once after release from the holder and behaves altogether normally except for possible signs of weakness; ether was administered only sufficiently to dull the operative pain. The sign + signifies slight reaction such as might be manifested by the animal lying on its side for a few minutes with somewhat dyspneic breathing. Severer but not dangerous symptoms are characterized by ++, indicating a moderate reaction. Finally, a certain number of animals recovered after manifesting most violent distress, which evidently had its origin in

18. Gay and Southard: Jour. Med. Research, 1908, xix, 17.

TABLE 1.—Results of Intravenous Injections of Horse Serum Following Courses of Instillations of Serum Into the Nose in Guinea-Pigs

1	2	3	Instillations*		Intravenous Injections†									11	12
			4	5	6	7	8 Third		9 Fourth			10 Fifth				11 Final Weight Gm.	12 Remarks
Pig No.	Sex‡	Weight at Outset, Gm.	Intervals Between, Days	Total Number	First§ Results	Second¶ Results	Results	Days After 2d	Results	Amt. Serum c.c.	Days After 3d	Results	Amt. Serum c.c.	Days After 4th		
1	♂	360	2	6	L +	L +++	L ++	73	L +	....	69	....	....	..	815	
2	♀	270	2	6	L 0	L ++	L ++	82	L 0?	....	69	L 0	0.88	37	810	Note in Column 10 more than double the usual toxic dose
3	♂	270	2	6	L +	L +++	.....	..	.....	....	...	....	....	..	?	Pig died through accident
4	♂	240	2	6	L +?	D	.....	..	.....	....	...	....	....	..	510	
5	♀	180	2	6	D	.....	.....	..	.....	....	...	....	....	..	360	
6	♀	180	2	6	L +	L ++	D	..	.....	....	...	....	....	..	660	
7	♀	510	2	6	D	.....	.....	..	.....	....	...	....	....	..	600	
8	♀	510	2	6	D	.....	... .	..	.....	....	...	....	....	..	660	
9	♀	270	4	6	L +	L +++	L +	39	L 0	....	93	....	....	..	780	
10	♂	300	4	6	D	.....	.....	..	.....	....	...	....	....	..	480	
11	♀	240	4	6	D	.....	.....	..	.....	....	...	....	....	..	480	
12	♂	180	4	6	L ++	.....	.....	..	.....	....	...	....	....	..	420	Died from accident
13	♂	180	4	6	D	.....	.....	..	.....	....	...	....	....	..	420	
14	♀	260	4	6	D	.....	.....	..	.....	....	...	....	....	..	?	
15	♀	300	5	6	D	.....	.....	..	.....	....	...	....	....	..	?	
16	♂	240	5	6	D	.....	.....	..	.....	....	...	....	....	..	?	
17	♂	240	5	6	L +++	L ++	L +	34	L 0	....	93	....	....	..	930	
18	♀	180	6	6	L +	L ++?	D	47	.....	....	...	....	....	..	780	
19	♂	210	6	6	D	.....	.....	..	.....	....	...	....	....	..	510	
20	♀	180	6	6	L +	.....	.....	..	.....	....	...	....	....	..	480	Died from accident

21	♂	210	14	6	L 0	L ++	L 0	75	L +++	1.1	36	....	....	..	780	Note in Column 9 the usual toxic dose is nearly trebled
22	♂	210	14	6	L ++	L +++	L 0	75	L 0?	1.13	101	....	....	..	1,000	Usual toxic dose trebled after 101 days. This and following pig bled next day for serum
23	♂	180	14	6	L 0	L +?	L 0	75	L +?	1.13	101	....	....	..	870	
24	♀	240	24	6	L +—++	D	.....	..	.....	....	...	....	....	..	780	
25	♀	270	24	6	D	.....	.....	..	.....	....	...	....	....	..	870	
26	♂	270	24	6	D	.....	.....	..	.....	....	...	....	....	..	840	
27	♀	360	2	12	D	.....	.....	..	.....	....	...	....	....	..	600	
28	♀	390	2	12	D	....	.....	..	.....	....	...	....	....	..	690	
29	♀	360	2	12	D	.....	.....	..	.....	....	...	....	....	..	570	
30	♂	420	1	12	D	.....	.....	..	.....	.. .	...	....	....	..	600	
31	♀	330	1	12	D	.....	.....	..	.....	....	...	....	....	..	570	
32	♂	300	1	12	L +	L +	L 0	80	D	1	93	....	....	..	930	Succumbs in 93 days after third intravenous injection to nearly 1 c.c. serum
33	♂	240	2	5	L +	D	.....	..	.....	....	...	....	....	..	570	
34	♀	210	2	5	L +?	L ++	D	70	.....	....	...	....	....	..	690?	
35	♀	210	2	5	D	.....	.....	..	.....	....	...	....	....	..	420	Serum instilled in both nostrils at each treatment
36	♀	300	14	5	D	.....	.....	..	.....	....	...	....	....	..	900	
37	♂	300	14	5	L 0?	L +++	D	34	.....	....	...	....	....	..	690	
38	♀	240	14	5	L 0#	D	.....	..	.....	....	...	....	....	..	750	
39	♀	360	14	5	D	.....	.....	..	.....	....	...	....	....	..	690	
40	♂	270	14	5	D	.....	.....	..	.....	....	...	....	....	..	740	
41	♀	300	14	5	L +	D	.....	..	.....	....	...	....	....	..	720	

* Amount of serum instilled at one treatment = 0.19 c.c.

† Usual amount of intravenous injections 0.38 c.c. In this and the following tables, + = slight reaction; +++ = severe reaction; ++ = moderate reaction; 0 = no reaction.

‡ In this column ♂ stands for male and . for female.

§ Sixteen days after last instillation.

¶ Twenty-four days after first injection.

First intravenous injection 40 days after last instillation.

mechanical obstruction to the respiration; these are indicated by +++.

Practically all our fatalities occurred within four minutes of completing the injection. Necropsy in every case but one showed the lungs in characteristic distention. Manifestly, if a guinea-pig fails to react to the first toxic injection it may be either because sensitization has not been achieved or because the animal is refractory. Therefore a second toxic injection is necessary and to this the animal, in the first case, should succumb.

Following the second toxic injection, subsequent injections were made at various prolonged intervals and with the use at times of an increased amount of serum, up to thrice that selected as the ordinary "toxic" dose.

A glance at the table shows (Column 6) that, of forty-one animals treated by the nasal instillation of serum, twenty survived the first toxic injection. Of these twenty, five gave no positive evidence of sensitization but all of these survived a second toxic injection or more. Twelve animals showed slight reaction to the first toxic injection. One of these died shortly after from trauma and one succumbed to the second toxic injection; all the rest survived it. Of the remaining three surviving pigs, two exhibited moderate and one severe shock. Inspection of the table shows that of seventeen animals submitted to a second toxic injection twenty-four days after the first, thirteen survived it with a general increase in intensity of anaphylactic response. Further repetitions of intravenous injection were marked, as a rule, by rapidly declining sensitiveness, whatever the amount of serum used. Possibly the construction of the table may be made clearer by definitely tracing the history of a single animal.

Thus, Guinea-Pig 2 was a female and weighed at the time of the first nasal instillation of serum 270 grams. She received six treatments at intervals of two days. Sixteen days after the last instillation the pig received an intravenous injection of 6 minims (about 0.38 c.c.) of normal horse serum. The animal survived this toxic injection with no perceptible evidences of shock, indicated by the cipher (0). The second toxic injection of 0.38 c.c. of serum given twenty-four days after the first, was survived after a moderately severe reaction. Eighty-two days after the second toxic injection a third was given resulting in moderate reaction. Sixty-nine days later still a fourth injection caused no certain reaction. After waiting thirty-seven days further the pig received a fifth intravenous injection, but now the amount of serum introduced was 0.88 c.c., more than twice the quantity employed in previous injections. There was no reaction whatever.

Study of Table 1 recalls the caution suggested by Rosenau and Anderson against drawing inferences from too meager material in immunization experiments. But even so small a number of observations as ours suggests certain reasonable conclusions. When animals treated precisely alike, as our Guinea-Pigs 1 to 8, manifest the widest possible difference in reactivity to a toxic injection of antigen, we are impressed with the importance of the individual biologic factor.

It seems probable from the evidence offered in Table 1, which is supported by many other experiments, that the biologic attitude respecting the antigen, induced in the guinea-pig by the nasal instillation of serum, is a labile state and may be changed by further similar treatment. Thus, of our animals 1 to 8, treated on alternate days with six doses of serum, five survived the first toxic injection, while three animals (27 to 29), treated twelve times on alternate days, all died with the toxic injection. In contrast to this biologic lability induced by absorption of small doses of antigen, we shall later contrast the vital stability achieved by animals receiving in addition large doses of serum. This important distinction has received the attention of other observers, notably Schultz, Weil and Gurd.

The Influence of Rhythm in the Sequence of Nasal Instillations

The results of experiments in this direction are summarized in Table 2. The observations are, of course, far too limited a number to serve as basis for any hypothesis. This table is constructed on the same lines as the preceding except as to Column 5. Here is shown the order of the nasal treatments. Thus, the first group of two pigs received instillations of serum in alternate nostrils on the first, fourth, fifth, seventh, tenth and eleventh days. Some special interest attaches to the last two groups of three animals each. In the first the order of differences in the intervals of treatment was 4, 3, 2, 1. In the second it was 1, 2, 3, 4. Although the same proportion of animals survived the first toxic injection in each group, there was a markedly intensified resistance in the surviving members (49 and 51), of the last group and it appears that further work on this line is desirable. It is hardly necessary to observe that this field of investigation seems intimately related to that which concerns the spacing of therapeutic vaccines.

The Effects of a Single Instillation and of Two Successive Instillations of Serum

The foregoing results led to the obvious inquiry as to what might be the least number of instillations of serum into the nose which could produce demonstrable reaction. Three minims (0.19 c.c.) of serum were dropped into the nose of each of four young guinea-pigs. Sixteen days later an intravenous injection of 6 minims (0.38 c.c.) caused no

TABLE 2.—The Influence of Rhythm in the Intervals Between Successive Instillations of Serum

1 Pig No.	2 Sex	3 Weight at Outset, Gm.	4 Number of Instillations	5 Sequence of Instillations, Days	Intravenous Injection									11 Final Weight Gm.	12 Remarks
					6	7	8 Third		9 Fourth			10 Fifth			
					First* Results	Second† Results	Results	Days After 2d	Results	Amt. Serum c.c.	Days After 3d	Results	Amt. Serum c.c.	Days After 4th	
42	♀	360	6	1, 4, 5, 7, 10, 11	D	.....	.....	..	.....	....	...	....	....	..	540	
43	♀	390	6	1, 4, 5, 7, 10, 11	D	.....	.....	..	.....	....	...	....	....	..	540	
44	♂	420	6	1, 2, 5, 7, 8, 11	L +	L ++	L +	67	.....	....	...	....	....	..	810	
45	♀	420	6	1, 2, 5, 7, 8, 11	D	.....	.....	..	.....	....	...	....	....	..	570	
46	♀	360	5	1, 5, 8, 10, 11	L +++	D	.....	..	.....	....	...	....	....	..	605	
47	♀	390	5	1, 5, 8, 10, 11	D	.....	.....	..	.....	....	...	....	....	..	540	
48	♂	240	5	1, 5, 8, 10, 11	L +	D	.....	..	.....	....	...	....	....	..	600	Both nostrils instilled at each treatment
49	♂	240	5	1, 2, 4, 7, 11	L ++	L +++	L +++	66	D	0.75	82	....	....	..	750	Succumbed to double the usual toxic dose
50	♀	210	5	1, 2, 4, 7, 11	D	.....	.....	..	.....	....	...	....	....	..	330	
51	♀	210	5	1, 2, 4, 7, 11	L +	L +++	L +	66	L +	0.5	57	L 0	0.75	31	780	Bled for serum 31 days after fifth intravenous injection. Instilled both nostrils

* Sixteen days after last instillation. † Twenty-four days after first injection.

disturbance whatever. When the injection was repeated twenty-four days later the pigs promptly died. The same result was obtained whether the serum had been instilled into one or both nostrils.

It having been shown that a single instillation of serum is ineffective—that it is, in physiological terms, a stimulus below the threshold of somatic irritability—it concerns us to know whether a second instil-

TABLE 3.—The Influence of Two Nasal Instillations of Serum at Intervals of One Day or More

1 Pig No.	2 Sex	3 Weight at Outset, Gm.	4 Intervals Between Intilla-tions, Days	Intravenous Injection 5 First Results	6 Second Results	7 Third Results	7 Third Amount Serum c.c.	7 Third Days After 2d	8 Final Weight
52	♂	360	1	L 0	L++	D	....	50	720
53	♂	390	1	L +	D	....	....	..	630
54	♂	360	2	L 0	D	....	....	..	690
55	♂	360	2	L 0	D	....	....	..	690
56	♂	300	3	L + ?	D	...	....	..	630
57	♂	275	3	L + ?	D	....	....	..	660
58	♂	270	6—*	L + ?	L 0 ?	D	0.5	28	630
59	♂	300	6—*	L 0 ?	L++	L +	0.63	80	930
60	♀	270	6	L 0 ?	D	....	....	..	690
61	♂	240	6	L +	D	....	....	..	600
62	♀	240	9	L 0 ?	D	....	....	..	630
63	♂	210	9	L 0 ?	D	...	.. .	..	540
64	♀	270	9—	D	.....	. ..	....	..	480
65	♀	270	9—	D	.... .	....	....	..	480
66	♂	210	12	D	... ..	....	....	..	420
67	♂	180	12	L 0 ?	D	....	....	..	540
68	♀	210	14—	L++	D	....	....	..	630
69	♂	240	14—	D	.....	....	....	..	450
70	♂	270	14—	L 0	D	.. .	....	..	630
71	♀	210	16	L +	D	....	....	..	600
72	♂	180	16	L 0 ?	D	....	....	..	600

* In Guinea-Pigs 58 and 59 interval between instillations above 3 hours less than 6 days. Note increased resistance of 59 with lapse of time. Thirty days after third intravenous injection 59 was bled for serum.

lation can summate its influence with the first so as to give rise to distinct reactions and, if so, whether the interval elapsing between the two stimuli is of significance. The outcome of our experiments in this direction is depicted in Table 3.

Of the twenty-one animals, four promptly died with the first intravenous injection of 0.38 c.c., sixteen days after the last instillation of horse serum. In these four the intervals between the two instillations ranged from somewhat less than nine to nearly fourteen days. That is, the periods were presumably sufficient for the production of free antibodies from the first instillation by the time the second was given. Of the seventeen pigs surviving the first toxic injection, four showed no discernible signs of reaction, while in thirteen the result was decidedly positive or doubtful. A second intravenous injection of 0.38 c.c. serum given twenty-four days after the first was survived by three animals. Pig 52 had shown no reaction with the first toxic injection, was moderately but not dangerously infected with the second but succumbed to a third, which followed the second in fifty days.

TABLE 4.—The Influence of Two Nasal Instillations of 0.19 C.C. Serum at Intervals of from Fifteen to Thirty Hours

1 Pig No.	2 Sex	3 Weight at Outset, Gm.	4 Intervals Between Instillations, Hours	5 Intravenous Injection. First Results	6 Intravenous Injection, Second† Results	7 Final Weight	8 Remarks
73	♀	270	15	D	..	420	
74	♀	300	15	L 0 ?	D	630	
75	♀	300	19	D	..	?	Intravenous injection = 0.25 c.c. serum
76	♀	270	20	L 0 ?	D	630	
77	♂	240	20	L + ?	D	570	
78	♂	240	25	L + ?	D	540	
79	♂	270	25	D	..	420	Lungs nearly collapsed at necropsy
80	♀	270	30	L + ?	D	690	
81	♂	270	30	L 0 ?	D	570	

Pigs 58 and 59 survived two intravenous injections. No. 58 succumbed to a third injection twenty-eight days after the second, the amount of serum injected having been increased to 0.5 c.c., one and one-fourth times the usual dose. Pig 59, which had reacted much more plainly than the preceding to the second toxic injection, did not receive the third intravenous injection until the lapse of eighty days, when it hardly reacted to the injection of 0.63 c.c. serum, one and two-thirds the usual amount. Thirty days after its third intravenous injection this pig was bled and its serum injected into normal animals with results that will be described later.

The general conclusions from this group of experiments are that two nasal instillations of serum separated by intervals of one to sixteen

days usually demonstrably modify somatic irritability and that such modification may be profound even when a large toxic injection gives negative results.

The curious protection manifested by Pig 52, which had received nasal treatments separated by about twenty-four hours, made advisable a more careful study of the effect of two instillations separated by small intervals of time. In Table 4 are condensed the results of experiments on nine animals, each of which received two instillations separated by intervals of from fifteen to thirty hours. Of the six pigs surviving the first toxic injection, three gave positive and three doubtful signs of sensitization; but none were made refractory. It is worth noting that on necropsy the lungs of Pig 79 were found nearly collapsed and that they *seemed* to expand slightly later.

It is plain from the foregoing that the fundamental question why certain animals become sensitized and others are made refractory by nasal instillations of serum is definitely answered neither by the spacing of the treatments nor by the rhythm of their succession, although these factors apparently have an important bearing on the results. On further reflection it seemed as though the varied results of different series of instillations might easily be accounted for. Thus, it is reasonble to suppose that the state of sensitization developed by any instillation of serum would be annulled by the next succeeding treatment, following the well-known conditions of desensitization, and that a subsequent toxic injection would be tolerated. According to this idea, when a series of instillations is given spaced so as to allow each treatment to exercise its full effect before the next is applied, the animals receiving an even number of treatments should all survive the first toxic injection, while those subjected to an odd number of instillations should succumb to the injections. This view seemed supported by the remarkable resistance exhibited by Pigs 21 to 23 of Table 1, which had received six instillations at intervals of fourteen days, compared with the hypersusceptibility of Pigs 36 to 41, which had received but five instillations. In experimental test of this theory it seemed reasonable to consider all instillations obviously falling within the incubation period of the first as a single treatment.

Twelve guinea-pigs, accordingly, were divided into four groups, each of which was given five instillations of serum spaced as shown in Table 5.

In Group 1, it was assumed that the four daily instillations acted essentially as a single larger dose. Twelve days after the last treatment, namely, on the sixteenth day, the final instillation was given. Sixteen days later the toxic injection was given and, according to

theory, should have been withstood; but it was fatal to all three animals. In Group 2, after three instillations we waited twelve days and then gave a fourth treatment on the fifteenth day. This treatment, according to theory, should have desensitized the animal so that the next instillation (in the case of two pigs given on the following and in the remaining animal after two days) should have sensitized the animals and led to fatal results from the first toxic injection. Such indeed was the issue, but it means nothing in view of the result in Group 1. The experiment in Groups 3 and 4 were identical except that sixteen days were allowed to elapse between the cluster of sensitizing instillations and the test instillations. It is noteworthy that of the two pigs surviving the first toxic injection one belonged to each group, though according to theory all of Group 3 should have lived and all of Group 4 should have died. Moreover, a consideration of the

TABLE 5.—Influence of Five Nasal Instillations at Varying Intervals

Group No.	Sequence of Five Instillations in Days	Number of Pigs Surviving		
		I. V. Inj. 1*	I. V. Inj. 2†	I. V. Inj. 3‡
1	1, 2, 3, 4, 16	0		
2	1, 2, 3, 15, 16	0		
	1, 2, 3, 15, 17	0		
3	1, 2, 3, 4, 20	1	1	0
4	1, 2, 3, 19, 20	0		
	1, 2, 3, 19, 21	1	0	

* First intravenous injection sixteen days after last instillation; 0.38 c.c.
† Second intravenous injection twenty-two days after first; 0.38 c.c.
‡ Third intravenous injection seventeen days after second; 0.5 c.c.

history of Pigs 24 to 26 of Table 1 militates against the theory. These animals had received six instillations of serum at intervals of twenty-four days, yet only one survived the first toxic injection. At the sixth instillation this pig, which had about half completed a period of pregnancy, showed active abdominal peristalsis, while the remaining two suffered severe asthmatic seizures.

It would appear from the foregoing account that, so far as the evidence goes, *the accepted principles of desensitization or antianaphylaxis cannot explain the survival of the first toxic injection by animals which have received a course of serum instillations by the nose.* Even could tolerance to the first toxic injection be accounted for as the result of previous desensitization, the explanation of the survival of the second toxic injection would be rendered possibly more difficult.

The Influence of Nasal Instillation of Serum on Guinea-Pigs Previously Fully Sensitized by Subcutaneous Injection

Such profound effects having been produced by administration of horse serum through the nose in normal guinea-pigs, it was desirable to ascertain if in fully sensitized animals reactivity to toxic injections could be modified by the same treatment. Rosenau and Anderson[16] had already pursued the same idea. They gave to fully sensitized pigs small subcutaneous injections of antigen and could not find any sign of tolerance being established. Similar negative results attended the efforts carried out by one of us in the work referred to.[1]

But since this phase of the subject seems to illustrate the conditions determining the rational use of vaccines in disease, and since there seems increasing confidence, especially among rhinologists,[19] in the therapeutic efficacy of local and general administration of antigens in what are apparently anaphylactic disorders, no opportunity should be lost to gather experimental data which might be pertinent to the problem.

Four guinea-pigs were given subcutaneous injections of 0.25 c.c. horse serum on June 8. July 15 to 19 they were subjected to certain losses of brain tissue without, as determined by controls, essential modification of sensitization. More than fifty days after the sensitizing injections, nasal instillation of serum was begun, each pig receiving twelve doses, two of the animals being treated daily and two on alternate days. Both of the animals receiving daily treatment began to show slight signs of general reaction with the second instillation, as manifested by respiratory râles, cough and labored breathing. With further treatment these reactions developed into very severe paroxysms of asthma which came on a few minutes after beginning instillation, lasted about half an hour and still showed effects after six hours.

With the last few instillations the asthmatic symptoms gradually failed, though signs of local irritation, as nose rubbing, persisted. In our previous work no general symptoms were produced in sensitized pigs by the nasal instillation of serum until about eight days had elapsed after the first instillation. In the foregoing cases general symptoms were elicited after twenty-four hours. It is a question whether the excision of a portion of the brain had anything to do with this shortened latent period.

The reactions of pigs instilled on alternate days pursued a similar course except that the first manifestations of general reactions did not appear until six days after beginning treatment.

These four animals were given intravenous injections of 0.38 c.c. of serum sixteen days after the last instillation and all promptly died

19. Goodale, G. J. L.: Ann. Otol., Rhinol. and Laryngol., 1914, xxiii, 278.

in respiratory anaphylaxis but with a marked absence, for the most part, of the general convulsions usually distinguishing such exitus.

From the well-known teachings of Besredka and others it might be supposed that a series of instillations of serum by the nose within the latent period of subcutaneous sensitization might "vaccinate" the animal so that it would tolerate, at least, the first intravenous injection of serum.

Two young guinea-pigs, which had suffered operative loss of part of the brain, but were otherwise normal, were given subcutaneously each 0.38 c.c. of horse serum and immediately thereafter 0.19 c.c. of serum were instilled into a nostril of each. Six instillations were given, in one case the treatment being repeated daily and in the other every other day. Sixteen days after the last instillation the pigs received intravenous injection of 0.38 c.c. of serum.

The result was fatal as in the previous group. It may be worth mentioning that the pig receiving daily treatment showed noticeable disturbance of respiration with the second instillation. So far as these experiments go they indicate that the nasal instillation of serum is capable neither of annulling subcutaneous sensitization which is fully developed nor of modifying the process of subcutaneous sensitization when administered during the incubation period.

Hereditary Transmission of the Anaphylactic State

Twenty-nine offspring from our guinea-pigs became available for observation. These fall naturally into three groups.

1. Seven pigs were born to four mothers that had each received subcutaneous injection of 0.25 or more of horse serum. The parents had been subjected to operations on the brain after sensitization. The offspring were tested by intravenous injection of 0.05 to 0.38 horse serum thirty or forty days after birth. All promptly died except one of a litter of three the mother of which had received her subcutaneous injection only thirty-four days before parturition, and therefore within the latter half of the period of gestation. Two of this litter promptly succumbed to the intravenous injection of 0.25 and 0.38 c.c., respectively, the third surviving the injection of 0.12 c.c. The litter was tested on the fortieth day of life. Of the seven animals in this group only one survived the first small intravenous injection.

2. Fourteen of the offspring were from four mothers which, after a course of five or six nasal instillations of serum, had tolerated from one to three intravenous injections of 0.38 c.c. of horse serum. One of the offspring was not tested until the eighty-third day after birth, when it was not affected by the intravenous injection of 0.38 c.c.

horse serum; that is, the passive sensitization had passed off. Two others from the same litter, tested on the thirty-first day, survived the injection of 0.25 and 0.06 c.c. serum, respectively, the former with very severe and the latter with moderate symptoms. But these pigs quickly succumbed to a second intravenous injection fifty-two days after the first. A similar result attended the second injection of all other pigs of this group which survived the first injection, receiving their second injection from twenty-four to eighty-seven days after the first. We must conclude that, for whatever reason the offspring resisted the first toxic injection, it was not because there was transmitted to them an ability to destroy the antigen before it sensitized their tissues. Of the thirteen offspring of this group receiving intravenous injections, usually of 0.25 c.c. or more of serum, within thirty-five days of birth, no less than five survived the first toxic injection.

3. Seven offspring were available from four mothers which had received a course of four or five nasal instillations. None of the parents had been given the toxic injection except two which had received 0.38 c.c. serum in a vein five days before the birth of their young. Five of the seven offspring survived an intravenous injection of 0.25 c.c. serum given nineteen to thirty-three days after birth. All succumbed to a second intravenous injection given seventeen days or more after the first.

It is obvious from the foregoing account that a higher degree of susceptibility is transmitted to their offspring by mothers that have been sensitized by subcutaneous injection than by those that have received a much larger quantity of serum intravenously following courses of nasal instillations. Still more marked is the comparative insusceptibility in the offspring of mothers which had been treated only by the instillation of serum into the nose, though they were subsequently found to be more or less refractory to toxic injections.

In his classic researches on inheritance of sensitization, Ehrlich found it necessary to limit his observations to the offspring of mothers that had already been fully sensitized before the beginning of pregnancy.[20] When the parent was sensitized after gestation had set in there was always chance that an active immunity might be conferred on the fetus. The offspring in our experiments were from mothers that had been serum-treated during the term of pregnancy. Nevertheless it is most improbable that the numerous survivals from the first toxic injection could have been due to an active immunity, since all the animals succumbed to the second toxic dose.

20. Morgenroth and Braun: Handbuch d. path. Mikroorg., 1913, ii, 2, p. 1155.

Experiments on Normal Guinea-Pigs Which had Received Intraperitoneal Injections of the Serum of our Immune Animals

Immunologists agree that the serum of an immune animal injected into a normal individual renders the latter passively anaphylactic to the antigen used. The higher the degree of immunity possessed by the donor the greater the number of antibodies in its circulation and the smaller the quantity of serum necessary to the transfer of sensitization.

Guinea-Pigs 22 and 23 of Table 1 sustained with practically no reaction a fourth intravenous injection of 1.13 c.c. horse serum each 101 days after the third injection. Twenty-four hours later these pigs were bled and their serums separated in a centrifuge. We are greatly indebted to our colleague, W. C. Mitchell, who kindly volunteered to carry out this phase of our work. The serum from each animal was at once injected into the peritoneal cavities of three normal pigs weighing about 240 grams each. In each lot of three normal pigs one received 0.5 c.c., one 2.0 c.c., and one 4.0 c.c. or 5.0 c.c. of serum.

Two days later the six pigs were given intravenous injections of 0.25 c.c. horse serum each. All the animals recovered; four had very slight reactions, but in the two which had received only 0.5 c.c. of serum in the abdomen the reaction was moderately severe. On the whole the anaphylactic phenomena differed widely from those usually witnessed, and resembled exactly the reactions manifested by sensitized pigs after excision of portions of the brain. The animal when released from the holder lies on its side with legs held stiff and involved in clonic tremblings; occasionally grotesque, tenanoid movements of the body occur; very minor, if any, respiratory disturbance is seen. Seventeen days later a second intravenous injection of 0.25 c.c. horse serum was given each of the six pigs. All died within five minutes except the two pigs which had received the smallest intraperitoneal injection (0.5 c.c.) and which had responded most strongly to the first toxic injection; these recovered, one after slight and the other after very severe reaction.

Eighteen days later these two animals received a third intravenous injection of 0.25 serum. Both died in four minutes.

According to current notions, these two pigs must have been sensitized by the intraperitoneal injection of 0.5 c.c. of immune serum and desensitized by the intravenous injection of 0.25 c.c. serum given two days later; they therefore recovered from the second intravenous injection seventeen days later, but were resensitized by it and succumbed to the third intravenous injection after eighteen days. It is not, however, clear why the much larger amounts of immune serum given the remaining four animals neither sensitized to a lethal anaphylaxis at the first intravenous injection nor protected them from the second toxic injection. Also, it seems extraordinary that so large an amount as 0.25 c.c. serum should not have sufficed to resensitize strongly the two resistant pigs at the first intravenous injection.

Anderson and Frost[21] have shown that the "allergin" contained in 3 c.c. of the serum from a sensitized guinea-pig (an amount capable of sensitizing a normal pig in twenty-four hours) can be neutralized by admixture of an amount of horse serum less than 0.01 c.c. It is therefore conjectural how far the sensitizing properties of our immune

21. Anderson and Frost: Bull. 64,, Hyg. Lab., 1910.

serum may have been modified by the massive injection of antigen given one day before.

Accordingly the experiment was repeated, using as donors Guinea-Pigs 51 of Table 2, which had received a fifth intravenous injection to the amount of 0.75 c.c. horse serum thirty-one days before, and 59 of Table 3, which had received its third intravenous injection, amounting to 0.63 c.c. horse serum, eighty days before. The serum from each donor was prepared as in the first case and injected intraperitonealy into three normal pigs having an average weight of 330 grams. From the first donor the quantities of serum injected were 0.5 c.c., 1.0 c.c. and 9.0 c.c. From the second donor the amounts injected were 0.5 c.c., 1.0 c.c. and 4.5 c.c. Twenty-three hours later each of the recipient pigs was given an intravenous injection of 0.25 c.c. horse serum. All recovered after very slight reaction, the response being most marked in the pig which had received 9 c.c. serum in the abdomen.

Sixteen days later these six animals were given a second intravenous injection of 0.25 c.c. horse serum. All died after manifesting, with one exception, unusual tendency to recover. The struggles of the pig which had received 9 c.c. of serum were unique in intensity and duration, persisting about eighteen minutes before the animal succumbed. Strange to say, at necropsy the lungs in this case were semi-collapsed.

The only work we know that bears on this subject is that recently published by Gurd, who insists on the "presence in the circulating blood of immune animals of bodies which are potent to induce the hypersensitive state when introduced into normal animals and also of bodies which if injected in sufficient quantities are able to render normal animals immune. . . . Subsequent inoculation [of an injected animal] in sublethal doses results in the stimulation of the production of a second order of ferments whose activity is directed more particularly toward the more complete cleavage of the toxic split product."[22]

Gurd concludes that the injection into normal guinea-pigs of small amounts of immune serum sensitizes, while injection of large amounts renders them refractory. It may be remembered that in our first experiment under this head exactly the opposite was noted. We fail to see how Gurd's main conclusion is justified by his protocols. Further discussion of this subject will be deferred for the present.

Specific Tissue Resistance as a Factor in Immunity

We have shown that a few applications of horse serum to the mucous membrane of the nose in the guinea-pig profoundly modifies

22. Gurd, F. B.: Jour. Med. Research, 1914, xxxi, 205.

the organism. That a toxic injection of serum following such a course of treatment should be attended by signs of sensitization or death is wholly to be expected from our present conception of immunity processes. When, however, as in Pigs 2, 21 and 23 of Table 1, the first toxic injection is not only negative but fails to render the animal fatally anaphylactic to a second intravenous injection given twenty-four days later, it is obvious that the animal has been in some way positively protected by a biological modification induced by the nasal instillations of serum.

Since it is seen in the case of Pigs 52, 58 and 59 of Table 3 that such protection may be manifested by so few as two instillations separated by an interval of less than six days, it would seem that the anaphylactic antibody as usually understood could have no essential part in the protection for, according to all evidence, such antibody is not yet formed.

In the preceding section we have shown that we may suspect the existence of something in the serum of highly immunized guinea-pigs which when introduced into the normal animal protects it from the usual reactions of passive anaphylaxis. Gurd has already dwelt on this conception, but an essential feature of Gurd's protective body is its late formation in the course of immunization. It is conceivable that the relatively refractory state of some of our animals should be produced by a specific protective substance developed as a reaction to even two nasal instillations of serum. In other words, we are confronted with the possible existence of two different offspring of biologic reactivity, one the familiar *allergic* antibody and the other an as yet undefined *resistance* antibody.

We have no intention at this time to assume, except as a working hypothesis, so far-reaching a conception.

But there is another possibility in support of which we will hazard a course of reasoning before offering experimental evidence.

It seems to us that immunologists have not laid due stress on the autonomy of the living tissues as a factor in immunity. Living tissues exhibit a marked resistance against digestion by proteolytic enzymes that actively attack them dead.[13] According to Wells[23] "snakes are nearly or quite insusceptible to snake venom. Cunningham found that the serum of cobras was devoid of antitoxic property, so that the immunity of snakes must be ascribed to an absence of cell receptors in their tissues with which their venom amboceptor can combine." Roosevelt,[24] from another point of view, makes observations differing only in detail. There is, then, no *a priori* reason for

23. Wells: Chemical Pathology, 1914, pp. 62, 147.
24. Roosevelt, T.: Through the Brazilian Wilderness, 1914, p. 9.

denying to the tissues extreme plastic power, but it remains to prove that modification of tissue resistance is a part of the condition of immunity. The following observations lend strong support to such a view. In his numerous experiments supporting the cellular theory of immunity, R. Weil found often an extraordinary difference in reactivity to the antigen to be exhibited by the two horns of the uterus in the sensitized guinea-pig; that is, the avidity of the same tissue for the antibody is widely variable.

In his latest paper Weil[25] holds that the antibody attached to the tissue cell must become "activated" by the latter before it becomes capable of causing anaphylactic reactions with the antigen, a view which recalls the hypothesis of Friedemann,[26] according to which the antibody exists as a zymogen which is activated by contact with antigen.

Schultz,[27] in his pioneer work on the anaphylactic reactions of isolated muscular tissues, found that,

> Guinea-pigs that have been rendered tolerant to large doses of serum by repeated injections of serum yield muscle that reacts much like that from sensitized animals even when 1 c.c. intravenous injections cause no more prominent body reactions than those observed in normal animals. . . .
>
> In time, however, this protective mechanism becomes less efficient and an injection of serum may cause anaphylactic symptoms in the intact animal.

In his careful and well-known work on the same subject Dale[28] confirms these findings, but in addition relates a curious experience which seems to us of great significance. According to Dale, when guinea-pigs immunized by multiple injections of serum were kept a long time (say three months) after the last injection, the uteri taken from such animals were nearly insensitive to the action of horse serum, although the controls showed evidence of abundant antibodies in their circulation.

This observation led us to test definitely on our animals the influence of the time element on anaphylactic reaction. Our Guinea-Pigs 21, 22 and 23 had each received a third intravenous injection of 0.38 c.c. of horse serum on a certain date without reaction. Thirty-six days thereafter Pig 21 was given a fourth intravenous injection, of which the amount was increased to about 1.1 c.c. The animal recovered, after nearly dying from the shock. The remaining two pigs were kept for 101 days before receiving their fourth toxic injection of 1.13 c.c. serum. This they withstood with scarcely noticeable signs of reaction.

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25. Weil: Jour. Med. Research, 1915, xxxii, 107.
26. Friedemann, U.: Ztschr. f. Immunitätsforsch., 1909, ii, 591.
27. Schultz: Bull. 80, Hyg. Lab., 1912.
28. Dale: Jour. Pharmacol. and Exper. Therap., 1913, iv, 167.

It was the slightness of the response obtained from the massive intravenous injection in these pigs which led to a test of the antibody content of their serum which has already been described. Gay and Southard lay stress on their findings that animals rendered "immune," usually by large spaced injections of serum, reacquire sensitivity and "eventually become quite as susceptible as do animals which have had a small initial dose, the incubation period varying directly with the amount injected on the first dose or doses. It seemed more correct then, to refer to these animals as 'refractory' rather than 'immune.' It was furthermore noted that the offspring of such refractory animals, born during a period of full refractoriness in the mother, were not themselves refractory, but sensitive to intoxication by horse serum."[29] These lines are quoted to show how utterly different are the results of our parallel experiments performed on animals receiving an initial course of treatment through the nose.

Similar results were obtained in comparing Pigs 58 and 59 of Table 3. Pig 58 had received a second intravenous injection of 0.38 c.c. serum without obvious reaction, but twenty-eight days later succumbed to a third injection increased to 0.5 c.c. Pig 59, which had otherwise been treated just like 58 and had reacted much more sensibly to the second toxic injection, was kept for eighty days and then received its third intravenous dose, which was increased to 0.63 c.c., but responded with only slight reaction. Unfortunately the basic idea of this discussion did not develop until most of our animals appropriate to the investigation had been disposed of. Nevertheless a glance at the tables will show that Pigs 9, 17 and 44 showed no signs of reacquired sensitivity to the intravenous injection of 0.38 c.c. horse serum after resting periods of two or three months. Pigs 18, 32, 49 and 52 died at the third or fourth toxic injection. It should be noted, however, that in two of these cases the amount of serum injected had been increased to 0.75 and 1.0 c.c.

The outcome of these experiments seems to controvert Weil's view[30] that the protecting agency in cellular immunity is the antigen contained in the tissues which, gradually disappearing, allows the cells finally to regain their sensitiveness. It would rather appear that metabolism of antigen within the cells enables the latter to assume a resistance to the toxic substance as an essential end result of the process of immunization. The inference is obvious that the existence of the immune carrier of disease is in some way based on such tissue resistance as has been experimentally illustrated above. The results obtained in the experiments last described recall data presented by

29. Gay and Southard: Jour. Med. Research, 1908, xviii, 407.
30. Weil: Jour. Med. Research, 1914, xxx, 347.

Rosenau and Anderson[16] some years ago. These authors treated guinea-pigs with serial subcutaneous injections of horse serum to total amounts of 30 to 60 c.c.

The animals were kept as long as 249 days when each received an intracerebral injection of about 0.2 c.c. horse serum. The pigs reacted slightly or not at all. Two hours later the animals were bled and from 5 to 12 c.c. of the serum was injected subcutaneously into normal pigs. These were tested intracerebrally as before and each showed slight to marked symptoms.

Hektoen[31] calls attention to the established experience that "in actively immunized animals immunity may persist after the new antibodies have passed out of the blood."

SUMMARY

When a few drops of horse serum are instilled into the nose in guinea-pigs and the dose is repeated from one to five times at intervals varying from one to twenty-four days, about one-half the animals survive an intravenous injection of horse serum given sixteen days after the last instillation.

This survival is sometimes without symptoms, but cannot be due to previous desensitization, for a second intravenous injection is frequently well borne twenty-four days after the first.

We have tried in vain to find the conditions according to which the course of nasal instillations produce, on the one hand, the hypersensitive or, on the other, the refractory state. The accepted principles of desensitization do not account for the facts.

When, after several intravenous injections of 0.38 c.c. of horse serum guinea-pigs fail to react, the dose may, in certain cases, be doubled or trebled with like result.

Contrary to the usual experience, we have found in two groups of refractory animals that a large intravenous injection was better borne after a resting period of 101 days and 80 days, respectively, than in controls after 36 and 28 days.

When the blood serum of these immune animals was injected intraperitoneally into normal guinea-pigs, the latter, when given 0.25 c.c. serum by the vein twenty-three to forty-eight hours afterward, usually reacted very slightly. Two animals injected with 0.5 c.c. of serum into the abdomen showed stronger reaction to the toxic injection than those given 4 or 5 c.c. Also, they withstood a second intravenous injection after seventeen days to which the rest succumbed.

31. Kektoen, L., in Musser and Kelly's Practical Treatment, 1911, i, 256.

As is well known, the offspring of mothers sensitized by a single subcutaneouus dose of serum are themselves highly sensitive to a small toxic dose of serum given within about six weeks.

In our animals treated by instillation only, five out of seven of the offspring survived the toxic injection.

It is a pleasure to express our indebtedness to Dr. W. C. Mitchell and Dr. R. G. Morrison for essential aid in these experiments.

1360 Vine Street—2261 Albion Street.

CHANGES IN THE P WAVE OF THE HUMAN ELECTROCARDIOGRAM *

C. H. GODDARD, M.D.
BALTIMORE

The most important observations in regard to the "P" wave of the human electrocardiogram have been those concerning its changes in mitral stenosis. Various workers, including Einthoven,[1] Krauss and Nicolai,[2] and Samjoloff,[3] have called attention to an increase in height and often also a broadening of the P wave in this disease. To quote the last:

> Wir fanden ebenfalls bei Mitralstenose Verstärkung und meist auch Verlängerung der P-Erhebung. (We found likewise in mitral stenosis an augmentation, and generally also a prolongation of the P wave.)

Since the increase in the height of the P wave has been thought due to the auricular hypertrophy resulting from the narrowing of the mitral ring, it would be reasonable to believe that auricular hypertrophy, the result of any other pathological change, would have the same effect. Hence it has been the custom at this station to consider high P waves as more or less indicative of auricular hypertrophy. In measuring the height of this wave we have considered the P-T, and disregarded the P-R, ratio. The T wave is normally higher than the P, i. e., the P-T ratio is normally less than one. And so in cases in which the T waves were of normal average height, and the P-T ratio equal to or greater than one in one or more derivations, the curve has been considered suggestive of auricular hypertrophy—increasingly so as more derivations were involved. When the T wave was inverted, actual changes in the P height have been used as criteria, and a P wave which to the eye was plainly higher than normal has been considered suggestive of auricular hypertrophy. When both P and T waves were very small and much below the normal size, a P-R ratio of more than one has not been held to mean hypertrophy; i. e., we have felt that

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* From the Medical Clinic of the Johns Hopkins University.

1. Einthoven, W.: Le Télécardiogramme, Arch. internat. de physiol., 1906-7, iv, 148.

2. Kraus, F., and Nicolai, G. F.: Ueber das Elektrokardiogramm unter normalen und pathologischen Verhältnissen, Berl. klin. Wchnschr., 1907, xliv, 812.

3. Samjoloff, A.: Ueber die Vorhoferhebung des Elektrokardiogramms bei Mitralstenose, München. med. Wchnschr., 1909, lvi, 1943.

hypertrophy should be considered only in those cases in which there was an actual as well as a proportional increase in the P height.

By way of checking up the relationship between the P wave and auricular hypertrophy, the electrocardiograms of thirty-seven cases of clinical mitral disease on file at this station were examined, in which auricular hypertrophy might reasonably have been expected. These included twenty of insufficiency only, fourteen of combined

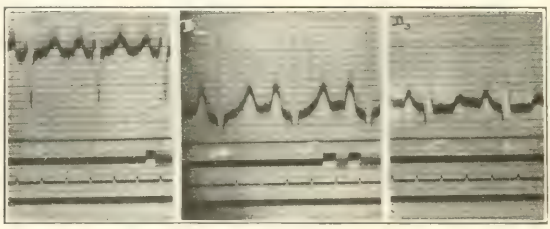

Fig. 1.—Case of mitral stenosis showing increase in height of P wave.

stenosis and insufficiency, and three of stenosis alone. The curves showed marked relative or actual exaggeration of the height of the P wave in twelve cases, or 33 per cent. It was moderately exaggerated in eight more, uncertainly exaggerated in five, and definitely small in the other twelve. Thus there was some relative increase in height in 54 per cent. of the cases studied. An example of the first group is shown in Figure 1, the electrocardiogram of R. K., white, aged 28. The diagnosis in her case was mitral stenosis and exophthalmic goiter.

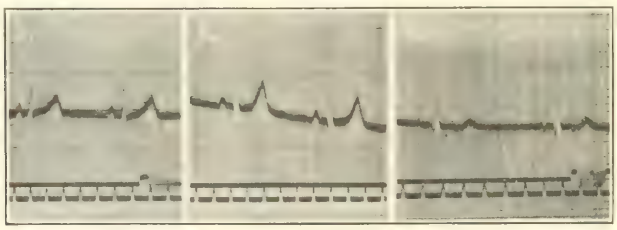

Fig. 2.—Case of mitral stenosis showing no increase in height of P wave.

Her heart measured, to percussion, 10 cm. to the left and 4 cm. to the right in the fifth and fourth interspaces, and the patient showed the typical signs of mitral stenosis. This is the type of case from which a diagnosis of auricular hypertrophy would be made unhesitatingly if a high P wave be taken as pathognomonic of this condition.

On the other hand, Figure 2 shows the electrocardiogram of a typical case of mitral disease in which there is no increase in the height of the P wave. The patient, F. S., white, aged 14, had a clinical

diagnosis of mitral stenosis and insufficiency and aortic insufficiency. His relative cardiac dulness measured 10 x 5.5 cm. in the fifth interspace. He had been admitted eight years previously with a diagnosis of chorea and mitral stenosis, so his cardiac changes were of long standing, and auricular hypertrophy to be expected. Yet the P wave is of normal size.

Five cases of the above series came to necropsy. Four of these showed auricular hypertrophy of varying degrees; the fifth slight dilatation of the right auricle only. This last and one other showed no increase in the size of the P wave; in the other three there was some increase, but this did not vary directly with the hypertrophy. Thus, one case of very slight hypertrophy showed a marked exaggeration of the P height, and one with much more marked hypertrophy a very slight exaggeration. It is to be noted that the only case in which the auricles were found tremendously dilated and hypertrophied, the mitral narrowing being very marked, was that of F. G., colored,

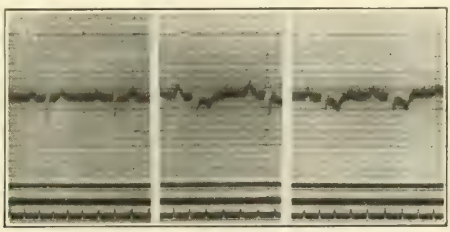

Fig. 3.—Case which showed at autopsy very marked dilatation and hypertrophy of the auricles. P wave increased in height in D_2 only.

aged 26, whose electrocardiogram is shown in Figure 3. It was taken three and a half months before death, the diagnosis being mitral stenosis and insufficiency. A high grade of auricular hypertrophy was probably then extant, as the cardiac symptoms were of three years' standing, yet, except for a relative enlargement in D_2 the height of the P wave is not increased, and its appearance would not suggest more than slight hypertrophy. It is noteworthy that the jugular tracings in this case showed a huge auricular "a" wave.

The matter was approached from another standpoint, and forty cases of electrocardiograms with high P waves were discovered in some seven hundred cases on file. The histories of the patients comprising this list were then examined. In four instances, the patients not being hospital cases, no history was available. Of the other thirty-six, sixteen, or 45 per cent., had a diagnosis of mitral disease. Thirteen others showed myocardial insufficiency, associated with syphilis, myocarditis, or chronic nephritis, auricular hypertrophy being a reasonable presumption. There were two cases of paroxysmal tachycardia, one

of pulmonary tuberculosis with positive Wassermann test, two of hyperthyroidism, one with an associated pulmonary tuberculosis, and two of psychoneurosis, one with a positive Calmette test and one uncomplicated.

The electrocardiogram of this last is shown in Figure 4. The patient, Mrs. C., housewife, had suffered from nervousness, "dazed condition," and chronic constipation since the birth of her first and only child six years previously. Examination showed a normal woman with normal cardiovascular findings and a rather small heart. Her relative cardiac dulness measured 7 x 3.5 cm. in the fifth interspace. The electrocardiogram shows relatively large P waves in all derivations, and might readily be diagnosed one of auricular hypertrophy.

A study of the foregoing figures is conducive to the belief that although mitral disease with presumptive concomitant auricular hypertrophy and large P waves are associated in a considerable percentage of cases, this association is not a constant one. And it would seem

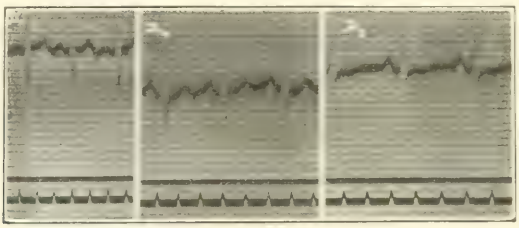

Fig. 4.—Case with clinically normal heart showing increase in height of P wave.

that while auricular hypertrophy probably in many instances exerts a causative influence, there must be many other factors which modify the height of the P wave. Indeed Einthoven[4] has demonstrated experimentally that increase in the height of this wave may be produced by cutting the vagus in dogs. He reports that vagal section may be followed by a rise in the P wave to nearly three times its former height. Plainly no gross organic change can take place in the auricles during the course of such an experiment, and we must look for other factors to explain the results recorded.

Changes in the height of the P wave may occur from beat to beat, as Figure 5, the electrocardiogram of a case of complete heart block of luetic origin, shows. The auricular wave immediately following a ventricular systole is comparatively small but there is a progressive increase in the height of the P waves until the next ventricular response occurs, when the cycle is repeated. It might be suggested

4. Einthoven, W.: Weiteres über das Electrokardiogramm, Arch. f. d. ges. Physiol., 1908, cxxii, 537.

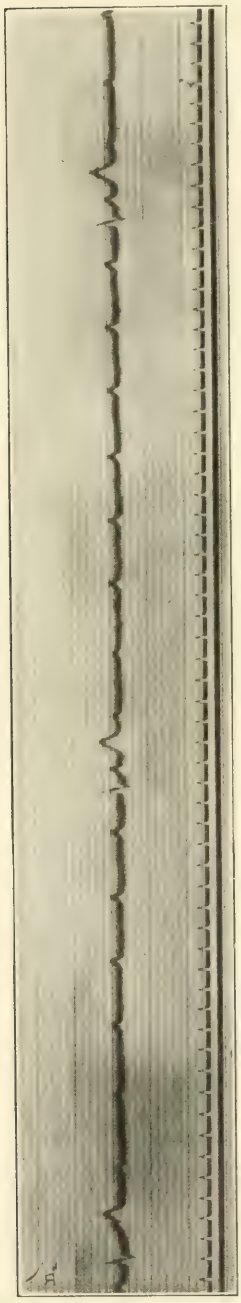

Fig. 5.—Complete heart block — luetic origin. Progressive increase in height of P waves between ventricular beats.

that each succeeding beat of the auricles, between ventricular beats, was working against greater pressure than the one immediately preceding, and that slight but progressively increasing functional dilatation was thus produced, such pressure and dilatation being relieved when the ventricle finally contracted. The idea of auricular *dilatation* exaggerating the height of the P wave is rather a novel one, and not well supported in the electrocardiographic findings in the two cases of our series which showed auricular dilatation at necropsy.

A review of the above findings would incline one to the belief that whatever the association between high P waves, mitral disease, and auricular hypertrophy, the dogmatic assertion that all cases of mitral stenosis will show high P waves, or that all cases of high P waves mean auricular hypertrophy, would be open to grave chance of error.

In reviewing the P waves of the seven hundred cases mentioned, the fact at once became apparent that inversion of this wave scarcely ever occurred except in the third derivation.

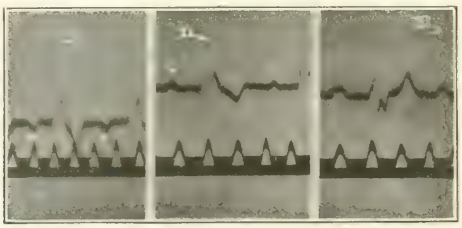

Fig. 6.—Same case as Figure 5, with normal sequence reestablished. P wave inverted in D_1, upright in D_2 and D_3.

It seems advisable to link inverted and diphasic P waves under one head, and, for simplicity, to speak of them all as "inverted," except where there is reason for differentiation. For the causative agents are apparently the same in both cases, and it is often difficult to classify a wave strictly under either heading. This is due to the fact that it may vary in appearance at different periods in the same curve, being now definitely inverted, now plainly diphasic.

To be specific, the P wave was inverted in the first derivation in but four cases of the entire series. Three of these were cases of *situs inversus,* in which such a phenomenon was to be expected. The fourth was the case of luetic heart block already referred to. The block passed off under treatment, and in an electrocardiogram taken later all waves were inverted in D_1, as in *situs inversus.* The relations in the second and third derivations, however, differed from those found in *situs inversus.* At this time the conduction time was prolonged (Fig. 6). Excepting the cases of *situs inversus,* this is the only case in which we have seen inversion of the P wave in D_1 without

inversion in the other two derivations. Of additional interest is the fact that the electrocardiogram of this patient while in a state of complete block showed a normal upright P_1 and P_2 with a diphasic P_3 (Fig. 7), while a subsequent tracing, of later date than Figure 6, has shown upright P waves in all derivations (Fig. 8). It is clear from this that changes in the direction of the P wave are not necessarily constant when once observed in a given patient.

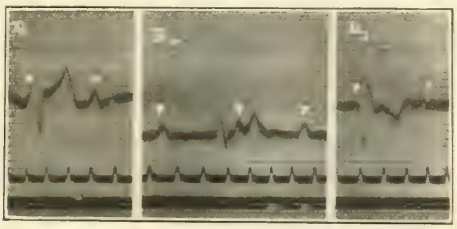

Fig. 7.—Same case as Figures 5 and 6. Complete block. P wave upright in D_1 and D_2, diphasic in D_3.

In a single instance, the P wave was diphasic in all derivations. The patient, R. S., colored, 35, was admitted in great decompensation with a right hydrothorax and a relative cardiac dulness extending 17 cm. to the left in the sixth interspace. The Roentgen ray showed a large heart lying transversely, with an unexplained mass (aneurysm?) in the region of the right heart. Diagnosis was myocardial insufficiency, arteriosclerosis, chronic nephritis. He was discharged improved with a relative cardiac dulness of 13 x 5 cm. in the fifth and fourth interspaces.

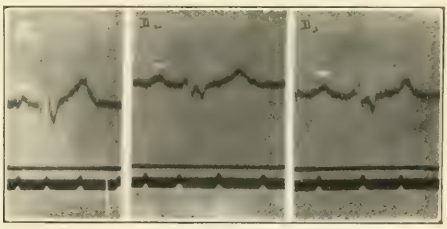

Fig. 8.—Same case as Figures 5, 6 and 7. Block has disappeared. P wave upright in all derivations.

The P wave was diphasic or inverted seven times in D_2, in patients with varying clinical diagnoses. In all of these it was inverted in D_3 also. In one case, R. Z., white, 23, no cardiac lesion was made out, the diagnosis being neurasthenia. The heart was enlarged, however, the relative cardiac dulness measuring 11.5 x 4.5 cm. In this instance the P wave varied in cycles, being upright, then diphasic, then inverted,

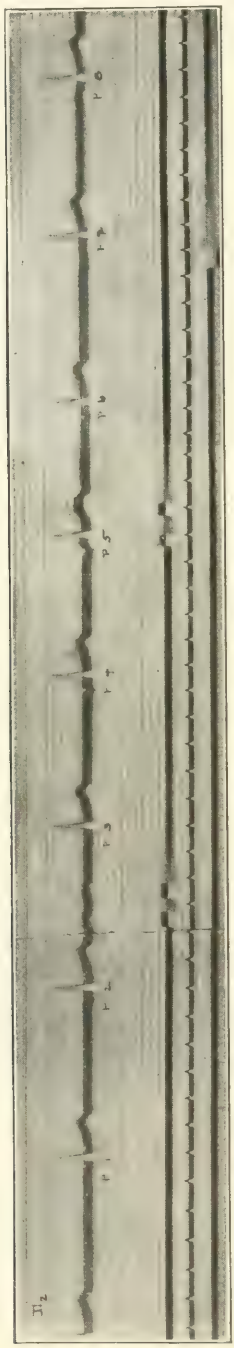

Fig. 9.—Case of neurasthenia with clinically large heart. P wave shows cyclic variation, being upright (1, 2), diphasic (3), inverted (4, 5, 6), diphasic (7), and finally upright again (8).

then diphasic, and finally upright again (D_2). The duration of a cycle was too long to ascribe it to respiratory effect. The electrocardiogram showed a pulse rate of 50, P_2 as described, diphasic P_3 and split R_3; otherwise normal (Fig. 9).

In seventy-five cases, about 11 per cent. of all on file, P_3 was diphasic or inverted. It is to be remembered that all cases of such changes in D_1 and D_2 numbered together but twelve. Examination of the clinical histories naturally showed that heart lesions were present in the majority of instances, since it is not the practice here to take electrocardiograms of many cases outside the cardiac type. Nevertheless, several cases were available in which diagnoses such as "chronic appendicitis and enteroptosis," "hyperthyroidism," "psychoneurosis and neurasthenia," "atrophic cirrhosis of the liver, pellagra," were made, and in which the cardiac condition seemed normal; although in most of these a large relative cardiac dulness, suggestive of emphysema or pulmonary tuberculosis, made auricular changes possible. At least

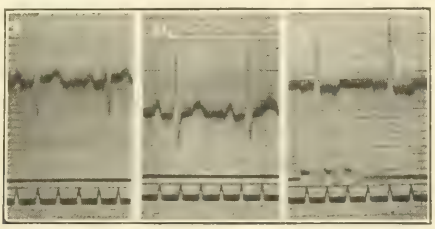

Fig. 10.—Case with clinically normal heart showing inverted P wave in D_3.

three cases, however, appear to be above suspicion. The first was N. U., white, a neurotic girl of 16, with a diagnosis of hyperthyroidism, and a normal heart. The second (Fig. 10) was a case of periodical paralysis in a young man of 23 with a relative cardiac dulness of 9 x 4.5 cm., and a clinically normal heart. The third, M. T., white, aged 54, was a case of chronic cholelithiasis following typhoid fever. His relative cardiac dulness measured 8.5 x 4 cm. in the fifth and fourth interspaces. Following operation he died of hemorrhage. Autopsy showed normal auricles.

Another case that came to autopsy should be noted. The patient, T. S., colored, aged 65, had had cardiac symptoms for a year and a half. The diagnosis was syphilis, mitral insufficiency, and chronic nephritis. The electrocardiogram, taken two weeks before death, showed beautiful inversion of P_2 and P_3. At autopsy the right auricle was slightly dilated, the left normal.

Seven of the thirty-seven mitral cases previously referred to showed inverted P_3. In only *one* of these was there any relative

enlargement of the P wave. This was in P. J., a woman of 20, diagnosis, mitral stenosis. There was progressive increase in the height of the R wave, the so-called "Right ventricular hypertrophy sequence."

An interesting feature was the discovery that 70 per cent. of all cases of inverted P wave showed the "left ventricular hypertrophy sequence," a progressive decrease in the R and increase in the S wave. These figures are not quite so impressive when we know that 47 per cent. of all the cases on file showed this sequence, but still they remain very significant. Twice only was the right ventricular hypertrophy sequence present. One of these cases was in P. J., referred to in the paragraph preceding. The other was that of a child of 8 years with a diagnosis of mitral stenosis and insufficiency, and aortic insufficiency.

It is worthy of note that in almost every instance in which neither ventricular sequence was present, interesting changes in the Q and S waves took place; i. e., a progressive increase or decrease in the size of one or both.

As to the causes of inverted P waves, their marked tendency to appear only in D_3, and their association with the left ventricular sequence, one can but speculate. Consideration of the facts at hand would lead to the impression that actual organic changes in the auricles are by no means necessary factors in the causation of the inverted P. Age may have an effect; only three of our series occurred in children (all with diseased hearts), but then the percentage of children's electrocardiograms on file is very small. It would seem, however, judging from the association between the inverted P and the left ventricular sequence, that the former, as the latter, will tend to appear more frequently with advancing age. The left ventricular sequence appears to be rather a normal finding in persons past middle life, i. e., it can hardly be considered necessarily indicative of pathological conditions, as Bridgman[5] has suggested. Similarly, although the inverted P is a less frequent finding, it would appear that it will tend to be found normally in a certain percentage of healthy adults.

Einthoven[6] has demonstrated that vagal stimulation may so reduce the height of the P wave that it becomes diphasic. Why then may not other influences not at present recognized produce similar changes, the auricles remaining uninjured? Perhaps vagal effects have some part in the clinical findings as well as the experimental, but hardly a prominent one, for vagotonie was not a feature of the cases studied. Pulse rates varied between wide limits, as did blood pressure. Cardiac position and respiratory influence may well each play a part. Other, probably more important, factors remain to be determined.

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5. Bridgman, E. W.: The Value of the Electrocardiogram in the Diagnosis of Cardiac Hypertrophy, THE ARCHIVES INT. MED., 1915, xv, 487.

6. Einthoven, W.: Weiteres über das Elektrokardiogramm, Arch. f. d. ges. Physiol., 1908, cxxii, 540.

CONCLUSIONS

1. Although mitral disease, with presumptive auricular hypertrophy, was accompanied in 54 per cent. of our cases by some relative increase in the size of the P wave, cases occur in which no such increase is found, and autopsy may reveal marked auricular hypertrophy which the electrocardiogram hardly suggested.

2. A considerable number, 45 per cent. in our series, of large P waves are associated with mitral disease, but such waves also occur in other conditions and in persons with clinically normal cardiac findings.

3. Inversion of the P wave is commonly found in D_3 only; in 70 per cent. of these cases in our series the phenomenon was associated with the "Left ventricular hypertrophy sequence."

4. Inverted P waves occur at all ages. The size and shape of the P wave may vary at different periods in the same curve, and an inverted P in one record may appear upright in a later tracing, and vice versa.

The causative factors in inversion of the P wave are yet to be determined.

I am indebted to Dr. E. W. Bridgman for many of my case records and other generous assistance.

SEVERE ANEMIA WITH REMARKABLE ELONGATED AND SICKLE-SHAPED RED BLOOD CELLS AND CHRONIC LEG ULCER*

JEROME E. COOK, M.D., AND JEROME MEYER, M.D.
ST. LOUIS

The unusual character of the blood findings in the case here recorded is alone sufficient to justify its report. When, however, we compare these findings, as well as the clinical history, with two similar cases previously reported, one by James B. Herrick, the other by R. E. Washburn, we are forced to the conclusion that we have in these three cases a group which belongs quite apart from anything heretofore described. We desire to present the history and findings in our own case, with the abstracts of the cases of Herrick and Washburn, and to show that the three have points of resemblance so characteristic and so constant that they cannot be explained as accidental. Our thanks are due to the above-mentioned authors for the opportunity of examining blood smears of their cases and for other courtesies.

AUTHORS' CASE.—*Personal History.*—O. B., a mulatto woman, aged 21, entered the Washington University Hospital, Nov. 3, 1914, giving the following history: She was born in St. Louis and had never been out of its immediate vicinity. She had had most of the diseases of childhood, and suffered at intervals, ever since she could remember, with "rheumatic" pains; the joints had never been swollen. Shortly after her marriage, two years ago, she developed "pus-tubes," which were removed a year later. At the age of 5 an ulcer broke out on her ankle, which healed in about a month, under treatment. This did not recur until two years ago when a similar condition developed which lasted three or four months and was healed with scarlet-red ointment and bichlorid packs. Ten months before entering the hospital the ulcer again appeared. It caused her no pain and has been treated in various ways.

Physical Examination.—Nov. 3, 1914. The patient was a light colored mulatto, with evidence of some loss of weight, conjunctivae and mucous membranes rather pale. The conjunctivae had a greenish tint. The rest of the physical examination, with the exception of the leg condition to be described, was not significant. There was a soft systolic murmur at the apex, transmitted to the left axilla, and heard over the left precordial area, the second pulmonic was louder than the second aortic and definitely accentuated. The systolic blood pressure varied from 105 to 125, the diastolic between 60 and 65. The lungs were clear, the spleen not palpable. On the left ankle, just above the internal malleolus was an ulcer 3 by 2 cm., the edges slightly indurated, the whole being of a light pink color. On the outer side of the ankle there was a scar 7 cm. by 3 cm. The skin in this region was dark and shiny.

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* From the Department of Internal Medicine, Washington University Medical School.

Course.—The ulcer soon healed under bichlorid dressings, has remained closed since, and now presents a smooth, irregular surface, not depressed, of rather glossy and somewhat transparent texture, not white, but rather of an ivory color through which a fairly close network of capillaries can be discovered on close examination. Since her discharge from the hospital, Dec. 3, 1914, the patient has gained in weight, feels stronger, and looks in every way improved. Our principal interest has, from the first, been centered on the blood picture. This will be taken up in detail later. Its unusual character made us feel that a full knowledge of the patient's family history possessed more than ordinary importance. We were able, by consulting several sources, to obtain fairly complete and accurate data.

Family History.—Patient's father, A. W., a mulatto, aged 54, has no knowledge of any blood relation except his children. The patient's mother died six years ago, aged 51, of breast cancer, twenty months after the disease was first noticed. The patient is the youngest of four children, there having been two boys and another girl. The first boy died at the age of 7. Up to a year before his death he was well nourished and apparently well; he then grew thin and sallow, had no cough, became progressively thinner, but was only confined to bed ten days before his death. The next child, a girl, died at the age of 22, of tuberculosis. She was healthy up to her fifth year, after which she became thin and sallow and suffered with pains in the extremities. The next child, a boy, died nine years ago at the age of 17. He was seen by Dr. E. W. Saunders, who furnishes the following note of the family at that time: "I found the children the victims of severe anemia. One of them died and then I lost sight of the family." The father says that this boy was strong and fat up to the age of 6, after which he began to get weak and very pale. None of the children except our patient had leg ulcer. The patient's mother was born in St. Louis and was never outside of the city. She was a healthy woman up to the time of her last illuness. The mother's family history reveals nothing significant, with the exception that there seems to have been a large admixture of white blood. The mother's sister and her daughter are living in St. Louis. They are light colored mulattos whose general appearance bear out the statement that they are in good health. Owing to some family enmity we have, as yet, been unable to persuade these two to submit to nearer examination nor even to permit blood smears to be taken.

During the patient's stay in the hospital numerous blood examinations were made. The most striking feature was the shape of the red cells in the fresh and stained preparations. The illustrations show the condition in general, though there are many variations in each type.

Many of the red cells, about one in three, on rough count, were elongated, oat-shaped or crescent-shaped, with the same color and structure as the normal red blood cells. Some of the crescent-shaped cells in the fresh drop would curl and bend as if they were unusually pliable. There was considerable variation in the size of the red cells and also many structures which seemed to be fragments of cells, as if some of the cells had been crushed. The rather uniform character of these fragments, however, suggests that they were not artefacts. Nucleated reds were always present and at times were quite numerous. With Romanowsky stains there was only slight polychromatophilia.

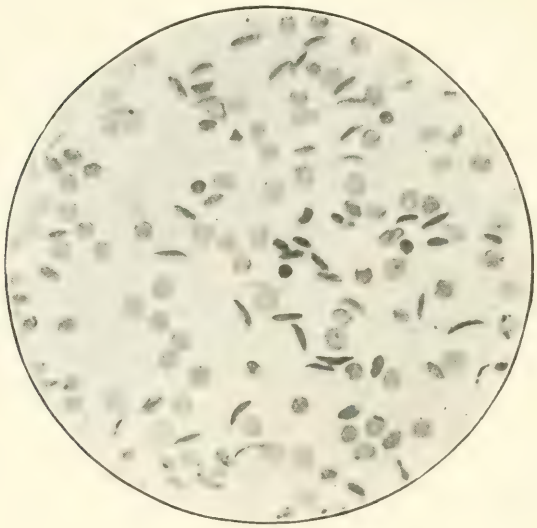

Figure 1

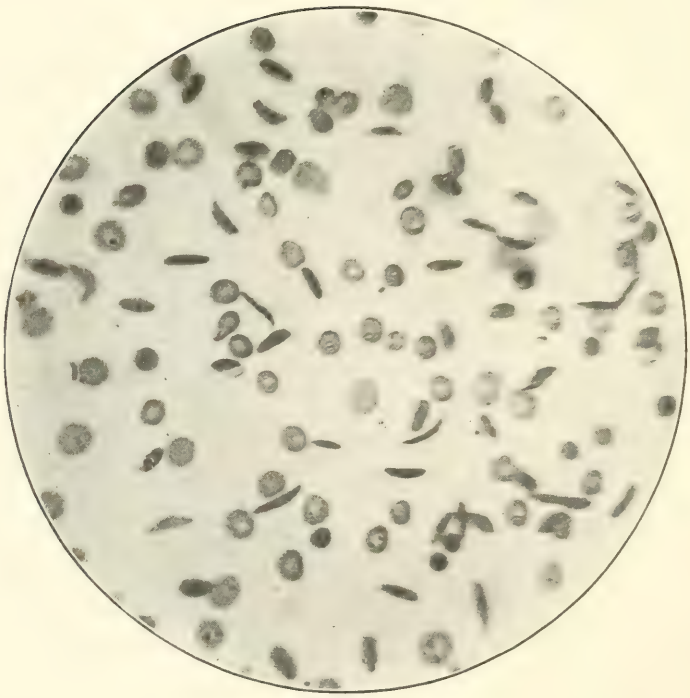

Figure 2

Figs. 1 and 2.—Elongated and sickle-shaped red blood cells.

The red cells varied in number between 1,800,000 and 3,100,000, usually about 2,500,000; there was at all times a leukocytosis of mild degree; the color index averaged slightly below 1, and there was a constant eosinophilia at the expense of the polymorphonuclear cells. The following counts show the variations and are selected from a large number, made at short intervals:

Cell Count and Hemoglobin

Date	Red Blood Cells	White Blood Cells	Hemoglobin, %
11/ 3/14	2,008,000	10,560	43
11/10/14	2,240,000	10,320	45
1/14/15	2,960,000	14,200	53

Differential Counts

Date	No. of Cells Counted	Normoblasts	Intermediates	Lymphocyte, %	Large Mononuclear, %	Polymorphonuclear, %	Eosinophil, %	Basophil, %
11/ 5/14	1,000	21	11	17.8	10.6	58.4	12.6	0.6
11/14/14	200	4	1	30.5	3	37.5	26	3
1/10/15	200	8	..	23	8	55	12	2

Before entering the Washington University Hospital, the patient had been in the Skin and Cancer Hospital. The blood examinations, made by Miss Florence Miller, clinical pathologist, show that the condition of the blood was about the same as in our experience. During the patient's stay in this institution search was also made for blood parasites, but without result.

The eosinophilia was subject to constant variation of rather wide range, and at one time we were able to follow a rise in the count from 3 per cent. to 26 per cent. within eleven days. The intense eosinophilia awakened the suspicion that the patient harbored a parasite. The stools were very thoroughly searched for evidence of this, but without avail. Nor did repeated examinations of the blood taken at all periods of the twenty-four hours, including centrifuged preparations of the citrated blood, reveal anything which could be interpreted as parasite or ovum. The Wassermann reaction was negative and an injection of salvarsan had no effect on the blood picture or the leg ulcer.

The suggestion that the abnormal shapes are artificial cannot well be proved. Some of the differences in shape depend on position, as

can be seen by watching a fresh preparation, but we could never see the change from oval or oat-shaped bodies to worm-like or sickle-shaped, or the reverse. We could not avoid getting the same forms, no matter how carefully the preparations were made, nor could we produce similar changes in normal or anemic blood from other cases. Dr. V. E. Emmel, associate professor of anatomy, who has made extensive studies of blood development and morphology, was much interested in this feature of our case, and made many investigations into possible explanations. His results will be published later.

The possibility of an inherited anomaly suggested itself. The fact that the three other children of the family had suffered from severe anemia encouraged investigation in this direction. Examination of the father's blood showed 4,500,000 red cells, 11,100 white cells, and 86 per cent. hemoglobin. Differential count showed: polymorphonuclears 68 per cent., lymphocytes 20 per cent., large mononuclears 8 per cent., eosinophils 4 per cent. The stained smear showed none of the peculiarities of the daughter's blood. Dr. Emmel, however, found some resemblance in the behavior of the blood. When preparations of the fresh blood were sealed under precautions which insured sterility and allowed to stand for several days at room temperature, the microscope revealed long, sharp projections and elongations from many of the red cells in the blood of both the father and the daughter. Dr. Emmel has not found similar appearances in the blood of any other person.

The following case was reported by Washburn:[1]

Washburn's Case.—The patient had been under observation since 1907; a negress, aged 25, a native and resident of Southwest Virginia all of her life. When admitted to the University of Virginia Hospital in 1910 she complained of weakness, soreness and pain in the left side, and a sore on left leg. When a child she had pneumonia and within the last five years has had chills and fever, supposedly malaria. On admission she had shortness of breath on exertion, night sweats and swelling about the wrists and ankles. There were occasional attacks of abdominal pain and indigestion.

The patient's father, an old man, has been in bad health for many years. The mother died twenty years before. The patient had four brothers, all of whom died while small, and four sisters, three of whom died in childhood, while one died recently of some brain trouble. The patient's illness dates back five years, when she noticed some dizziness, shortness of breath on exertion, and swelling about the wrists and ankles. At the same time she knocked some skin off the left shin, resulting in an ulcer. Some time later an ulcer started on her right leg, though she remembered no injury. The patient was operated on for gall stones in 1908. In April, 1909, the following blood examination was recorded: Red cells 2,000,000; white cells, 11,000; hemoglobin (Sahli) 50 per cent. Differential white count was as follows: Plymorphonuclear 63 per cent., small mononuclear 25 per cent., large mononuclear 2 per cent., eosinophil

1. Washburn, R. E.: Peculiar elongated and sickle-shaped red blood corpuscles in a case of severe anemia, Virginia Med. Semi-Monthly, 1911, xv, 490.

4 per cent., mast cells 6 per cent. The red cells showed poikilocytes in a variety of shapes, the most common being of a crescent shape. Washburn sums up his case as follows:

The case is one of severe anemia, characterized by a peculiar poikilocytosis, oligocythemia and a color index less than one, together with nucleated red corpuscles, myelocytes and an increase in the percentage of eosinophils. Among the suspected causes were syphilis and intestinal parasites, but both of these seem to be disproved. There is no definite history of syphilis, and the iodids, though given until the physiological effect was produced, had no apparent influence on the anemia or on the leg ulcers. No intestinal parasites capable of producing the trouble were found on examination of the stools either before or after the administration of thymol. The facts do not seem to warrant diagnosis as to the cause of the condition.

The following is abstracted from the case of James B. Herrick.[2]

Herrick's Case.—The patient, a negro of 20, had been in the United States three months; his former residence was in the West Indies, where he was born. He was one of four children, all the others being healthy. At the age of 10 the patient had had yaws, which was common in his locality. The disease lasted about one year and was characterized by ulcers on the legs, described as pustular with scabs, the scars of which remained. For about a year previous to coming under Herrick's observation he had noticed some palpitation and shortness of breath, and for about three years had had a disinclination for exercise. On landing in New York, September, 1904, he had a sore on one ankle which healed with tincture of iodin, leaving a scar similar to those on limbs. The patient came to Herrick for treatment of a "cold." Physical examination showed a young man, well developed physically, a tinge of yellow in the sclera and pale mucous membranes. The cervical, axillary, inguinal, and epitrochlear glands were definitely enlarged. The scars to which he referred were nearly all located on the legs and thighs, perhaps twenty in all. They were rounded or oval, sometimes of irregular contour, the edges clean cut; some were like tissue paper or thin parchment to the touch and were lighter in color than the surrounding skin. They were strikingly like scars often seen as a result of syphilis. A faint systolic murmur was heard over the base of the heart and to some extent over the apex. The blood examination on Dec. 26, 1904 was: red cells 2,880,000; white cells, 15,250; hemoglobin 50 per cent. Differential count, polynuclear 72 per cent., small mononuclear 15 per cent., large mononuclear 7 per cent., eosinophil 5 per cent. Nucleated reds were common. The shape of the red cells was very irregular, but what especially attracted attention was the large number of thin elongated sickle-shaped and crescent-shaped forms. These were seen in fresh specimens no matter how the blood was spread on the slide; they were likewise always present in the fixed specimens. Under treatment the blood improved in quality, but there was still a tendency to the peculiar crescent shapes in the red cells, though this was not so noticeable as before. Nucleated reds were present. Eosinophils made up 5 percent of the total white cells. On March 7, 1906, a blood count was made that showed red cells 2,700,000; white cells 30,500; hemoglobin 55 per cent. Differential count; polynuclear 58 per cent., small mononuclear 22 per cent., large mononuclear 12 per cent., eosinophil 7 per cent., myelocyte 1 per cent. The elongated and spindle forms were numerous and there were many nucleated reds. The patient passed from observation in 1907 in about the same state of health as before.

Careful search of the literature has been made for other similar cases or for any blood picture resembling that of our case, but we

2. Herrick James B.: The Archives Int. Med., 1910, vi, 517.

have discovered no text-book or article which refers to elongated and sickle-shaped cells such as we have described. A search of this kind must of necessity be incomplete; it adds some testimony, however, to the fact of the extreme rarity of this condition. The only accounts which we have found and which, though seemingly unlike our case, may have some relation to it and are for that reason mentioned here, have been published by Bishop[3] and Dresbach.[4] The former reports a patient 70 per cent. to 80 per cent. of whose red blood cells showed "a more or less well marked elliptical outline." Most of the elliptical cells measured 13 by 5 microns. The red blood cells numbered 5,400,000, hemoglobin 110 per cent. The sister's blood showed the same condition of the cells and her red blood cells numbered 5,000,000 with 100 per cent. hemoglobin. Four other members of the immediate family showed a normal blood picture. We are indebted to Dr. Bishop for the privilege of examining blood smears from his case. Dresbach reports a similar case in a mulatto, aged 22. There were 5,000,000 red blood cells, about 90 per cent. of which were more or less oval. The greatest variation showed a minimum diameter of 3.9 microns, with a maximum of 10.7 microns. A brother had normal corpuscles. In both cases the general health was good with normal red and white counts and hemoglobin and an absence of nucleated red cells.

It will be seen that there is a striking similarity in both the blood picture and the clinical history of our case with those of Washburn and Herrick. All three of the patients were of negro blood; all three suffered from peculiar, indolent, recurring leg ulcer; in all three the anemia was sufficiently severe to cause dyspnea on exertion, and in all of the cases there was a peculiar discoloration of the sclerae, which in our case and that of Washburn is described as a "greenish tinge" and which Herrick notes as "a tinge of yellow"; in no case could enlargement of the spleen be made out; responsibility for the condition could not be placed on syphilis or a parasite, although a careful examination was made in each case for such a possible etiological factor.

The uniformity of the blood abnormality is even more striking, the individual differences being of degree rather than of kind and depending no doubt to some extent upon the time at which observations were made.

The red cells in the three cases, in the numerous examinations made, varied between 1,800,000 and 3,000,000 with a striking tendency to remain around 2,500,000. In all of these cases there was a leuko-

3. Bishop F. Warner: Elliptical Human Erythrocytes, THE ARCHIVES INT. MED., 1914, xiv, 383.

4. Dresbach, Melvin: Science, 1904, xix.

cytosis of more or less marked degree, and in all cases there was an eosinophilia of distinct but varying degree. A varying number of nucleated red cells was present in all cases, in no case were they rare or difficult to find. A glance at the blood slides leaves no doubt as to the identical character of the elongated and sickle-shaped red cells. The percentage of these cells was greater in our case than in the other two, but even in these it was considerable.

We wish to express our thanks to Dr. George Dock for the privilege of studying the case and for his suggestions in the preparation of our work.

A STUDY OF THE HEMOLYTIC ACTIVITY OF THE SPLEEN IN PERNICIOUS ANEMIA *

OSWALD H. ROBERTSON, M.D.
BOSTON

There is a certain amount of evidence that the increased blood destruction occurring in pernicious anemia is in some way associated with an abnormal functioning of the spleen, since removal of this organ brings about a marked reduction in the quantity of blood destroyed. This reduction has been found in almost every instance where the blood destruction was studied before and after splenectomy.[1] Eppinger and Ranzi[2] and the author,[3] who report the two largest series of cases, found that the greatly increased hemolysis present before operation showed a constant and marked decrease afterwards, reaching normal in all but two or three cases. Yet the manner in which the spleen acted to produce increased blood destruction is as yet undetermined.

The literature contains comparatively few observations on the hemolytic activity of the human spleen either in normal or pathological conditions. Benard[4] tested salt solution extracts of two freshly excised normal human spleens and obtained negative results. In a case of severe hemolytic anemia with marked jaundice, Antonelli[5] was unable to demonstrate any hemolytic activity with a saline extract of the freshly excised spleen. Port[6] studied the spleen in one case of pernicious anemia immediately after operation, using both alcoholic and ethereal extracts. His results were entirely negative. McPhedran,[7] however, working with ethereal extracts, found a substance in the spleen obtained at postmortem from a case of pernicious anemia, which possessed a strong hemolytic action. This hemolysin, which belonged to the group of unsaturated fatty acids, could, however, be extracted from the liver and intestinal wall and was also found in normal organs other than the spleen.

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* Submitted for publication June 8, 1915.

* From the Pathological Laboratory of the Massachusetts General Hospital.

1. The quantitative estimation of urobilin in the stool was used as an index to the degree of blood destruction.

2. Eppinger, H., and Ranzi, E.: Mitt. a. d. Grenzgeb. d. Med. u. Chir., 1914, xxvii, 796.

3. Robertson, O. H.: The Archives Int. Med., 1915, xvi, 429.

4. Benard, H.: Thèse de Paris, 1913.

5. Antonelli: Progressive Med., June, 1914, p. 327.

6. Port, Fr.: Berl. klin. Wchnschr., 1914, li, 546.

7. McPhedran, W. F.: Jour. Exper. Med., 1913, xviii, 527.

Several writers have suggested the possibility that the abnormal amount of hemolysis occurring in pernicious anemia may take place in the portal system, first on account of the lack of any evidence of hemolysis in the peripheral circulation, and second, because the chief deposit of iron is found in the liver. In a case of pernicious anemia with splenectomy, Eppinger[8] tested the hemolytic properties of the blood obtained from the splenic vein at operation with a negative result. Huber[9] in a similar case tested the resistance of the unwashed red blood cells from the splenic vein against hypotonic salt solutions. He found no difference between the splenic vein blood cells and the cells from the peripheral circulation.

In view of the few observations which have been made on this subject, it seemed desirable to record the results of a study of the freshly excised spleen and splenic vein blood in six cases of pernicious anemia recently in which the patients were operated on at the Massachusetts General Hospital for splenectomy.

SPLEEN

A histological study of the spleen in these cases was practically negative. There was no abnormal phagocytosis, no pigmentation, nor any other evidence of increased hemolysis.

The saline extract for the following experiments was made by adding to finely ground up fresh spleen pulp, twice its volume of normal salt solution. The mixture was then agitated in a mechanical shaker for one hour and finally centrifugated at high speed till no further sediment could be obtained. A very small quantity of red cells—0.05 c.c. of a 2 per cent. suspension—was added to varying quantities of this extract, and after two hours at 37 C., the mixtures were centrifugated and read. There was no hemolysis either with the patients, or with normal cells.

Acting on the assumption that if conditions similar to those in the body were maintained, the result might be different, in the next case the spleen was kept warm and the process maintained at 37 C. throughout. Under such conditions there was likewise no hemolysis. Benard stated in his experiments with dogs that he obtained a splenic extract actively hemolytic for the animal's own cells only when he perfused the spleen thoroughly with salt solution beforehand. Perfusion of the spleen in a third case made no difference, nor did keeping the extract for several days, which according to Widal Abrami and Brule[10] tends to increase the hemolytic properties of the spleen extract.

8. Eppinger, H.: Berl. klin. Wchnschr., 1913, 1, 2409.
9. Huber, O. R. C.: Berl. klin. Wchnschr., 1913, 1, 2179.
10. Widal Abrami and Brule: Bull. Soc. d. hôp. de Paris, 1912, No. 13.

An alcoholic extract was next tried. This was prepared by shaking finely ground up spleen pulp with 95 per cent. alcohol for several hours. The supernatant fluid was then decanted, evaporated to dryness, and the residue taken up with salt solution. This extract also failed to hemolyze red blood cells.

On account of Port's negative finding with the ethereal extract of a freshly excised pernicious anemia spleen, a portion of one spleen was extracted with ether. This extract showed an intensely hemolytic action, though not quite as strong as that found by McPhedran in the pernicious anemia spleen at postmortem. McPhedran's findings make it apparent that the extraction of the fatty hemolysins offers very little hope of differentiating the pernicious anemia spleen from the normal spleen.

This absence of any evidence of hemolytic activity characteristic of the pernicious anemia spleen, coincides with the negative histological findings. It is still possible that the spleen does possess a hemolysin, but that the methods used in attempting to extract it are unsuitable to demonstrate its presence.

SPLENIC VEIN BLOOD

Blood from the splenic vein was obtained at operation by aspiration into a syringe before the pedicle of the spleen was ligated. Owing to the difficulties in technic, the blood in two cases began to clot in the syringe, or so soon after drawing that it was not available for all the tests.

Hemolytic Properties.—The plasma in the three cases in which the blood was obtained before clotting occurred, showed free hemoglobin; in two the plasma was definitely pink, in the third hemoglobin was evident spectroscopically. The presence of hemolysis here, however, can easily be accounted for as a result of manipulation during the delivery of the spleen from the abdominal cavity. Hemolytic experiments were made in two cases with the serum, using both the patient's own blood cells and normal cells, and as controls, serum taken from the patient's arm vein immediately before operation and serum from a normal person. Results were entirely negative and the addition of guinea-pig complement made no difference.

Morphology.—Blood counts varied in their relations to those of the peripheral blood. One case showed a considerably increased number of red blood cells in the splenic vein, the other showed somewhat fewer. The white counts were both higher in the splenic vein, while the differential counts were practically identical with those of the peripheral blood. These variations from the peripheral blood cannot be relied on as representing actual differences, on account of the factor

of error due to the above mentioned manipulation, during which process a larger number of cellular elements than normal may have been squeezed into splenic vein.

The appearance of the red blood cells was identical with that shown by the peripheral blood, with the exception that in one case there was a greater percentage of reticulated cells in the blood from the splenic vein. Blood platelets were strikingly abundant in spite of the fact that they were much diminished in the peripheral circulation. The fact that the spleen normally is a great storehouse for blood platelets, however, coupled with the factor of manipulation at operation, may well explain their great increase in the splenic vein at this time.

Resistance of the Red Blood Cells.—The only positive finding which could not be explained as a result of operative procedure, was the decreased resistance of the red cells from the splenic vein. This was constant in the three cases tested. For controls the patient's blood taken immediately before operation and normal blood were used.

	Hemolysis Beginning at Pct. Salt Sol.	Hemolysis Complete at Pct. Salt Sol.
Case 1.—Splenic vein blood	0.525	0.325
Peripheral blood	0.45	0.30
Control normal blood	0.45	0.325
Case 2.—Splenic vein blood	0.675	0.325
Peripheral blood	0.425	0.30
Control — normal blood	0.425	0.325
Case 3.—Splenic vein blood	0.675	0.35
Peripheral blood	0.525	0.35

In Case 2 the hemolysis, beginning at 0.675 per cent. was very faint and continued faint to 0.425 when it increased markedly. Case 3 showed well-marked hemolysis beginning at 0.675. In this case the blood was obtained from the spleen five to ten minutes after excision, which may account for a greater decrease in resistance than was shown by the other two cases. Huber's negative result might be accounted for by the fact that he did not wash the blood cells before testing their resistance, since unwashed cells are protected to a certain extent by the blood plasma present.

Banti[11] obtained blood from the splenic vein at operation in two cases, one a case of hemolytic splenomegaly and the other a splenic vein thrombosis. Both showed a decreased resistance of the cells from the splenic vein. Banti also compared the resistance of the splenic vein blood cells with that of the peripheral cells in dogs and found a slight but constant lowered resistance in the cells of the splenic vein. This difference in resistance was much increased after the

11. Banti: Semaine méd., 1913, p. 313.

injection of a hemolytic serum, suggesting that a possible normal function of the spleen had become markedly hyperactive. It seems not improbable that the decreased resistance found in the cells of the splenic vein in these cases of pernicious anemia, which was even greater than that in Banti's dogs, may have the same significance, i. e., an evidence of injury received by the cells during their passage through the spleen due to some toxic substance elaborated by that organ. These injured cells would then be more easily broken down in other parts of the body. In this way the absence of any direct hemolytic action of the spleen could be accounted for.

It was thought possible that this change in resistance might be duplicated in vitro by allowing the cells to remain in contact with splenic extract. The patient's peripheral blood cells as well as normal cells were incubated for two hours with the saline extract, then washed and their resistance to salt solution tested. No change in resistance was observed. Neither were they less resistant to a hemolytic serum after this procedure.

SUMMARY AND CONCLUSIONS

1. In a study of the hemolytic activity of the fresh spleen obtained at operation, from six patients with pernicious anemia, it was found that extracts of the spleen pulp made with both normal salt solution and alcohol, possessed no hemolytic properties. An ethereal extract, on the other hand, showed a marked hemolytic action. However, the hemolytic substance contained in this extract belonged to the group of unsaturated fatty acids and has been obtained in equally strong concentration from the normal liver and intestine.

2. The only change in the splenic vein blood obtained at operation, which could not be accounted for by manipulation, was a decreased resistance of the red cells. These cells were definitely less resistant than those of the peripheral circulation in the same individual and in normal controls.

3. The above findings seem to exclude any gross or easily demonstrable manifestation of hemolytic activity on the part of the spleen. However, the decreased resistance of the red cells in the splenic vein, would suggest that the spleen in pernicious anemia elaborated some toxic substance or enzyme which so injures the blood cells during their passage through that organ that they are more susceptible than normally to destruction elsewhere. It seems probable that in some other part of the body there is elaborated an abnormal hemolysin or an abnormally acting normal hemolysin.

A BACTERIOLOGICAL AND CLINICAL STUDY OF THE NONTUBERCULOUS INFECTIONS OF THE RESPIRATORY TRACT, WITH SPECIAL REFERENCE TO SPUTUM CULTURES AS A MEANS OF DIAGNOSIS

PAPER I.—INTRODUCTION, METHODS, GENERAL RESULTS OBTAINED, ORGANISMS FOUND AND THEIR RELATIONS TO THE VARIOUS INFECTIONS, AND A CONSIDERATION OF THE VALUE OF SPUTUM CULTURES *

JOHN A. LUETSCHER, M.D.
BALTIMORE

The present series of papers dealing with infections of the respiratory tract is not so much the product of a set problem, as an evolution of some work started four or five years ago. At that time, during an investigation to determine the comparative virulence of the pneumococcus in the sputum of lobar pneumonia at various stages of the disease, with special reference to crisis,[1] it became necessary to isolate the strains of pneumococci used, by means of sputum cultures on blood agar plates. I was then impressed with the ease with which this method could be used as a means of diagnosis in pulmonary infections other than pneumonia.

The first case under observation was that of a woman with acute bronchitis who had no previous history of cough or any disease pointing to an infection of the chest. The expectoration was typical, green, mucoid, and I was surprised on the next day to find a beautiful plate, consisting of a pure culture of green pneumococcus colonies. My conception of the pneumococcus was a narrow one. The pneumococcus in the chest to me meant pneumonia.

The next case was that of a robust middle-aged man, who had contracted a cold, following which he was suffering with severe fits of coughing occurring several times a day, at which times he would bring up fairly large, tenacious, green lumps of sputum. An examination of the chest showed marked impairment at the right base with

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* From the Medical Clinic of the Johns Hopkins Hospital.

* Other papers in this series: Paper II. Acute and Chronic Bronchitis, Bronchopneumonia, and Lobarpneumonia; Paper III. Chronic Nontuberculous Infections of the Chest with Special Reference to the Pneumococcus; Paper IV. Acute Coryza, Acute and Chronic Sinusitis, Acute and Chronic Tonsillitis, Acute Laryngitis; Paper V. Whooping-Cough.

1. Luetscher, J. A.: The Comparative Virulence of the Pneumococcus in the Sputum of Lobar Pneumonia at Various Stages of the Disease, with Special Reference to Crisis, Jour. Infect. Dis., 1911, ix, 287.

modified tubular breathing and showers of râles. The Roentgen ray showed an infiltration of this area, with the diaphragm apparently hooked up as though by adhesions. The Roentgen ray diagnosis was tuberculosis. Tubercle bacilli were never found. This condition persisted for two months. The physical signs then cleared up, the Roentgen ray no longer showed any infiltration, and the diaphragm was in normal position. There had never been fever over 99. During the entire illness the patient felt well, so well that he wanted to take his accustomed walks of miles in the country. The sputum cultures gave pure plates of typical pneumococcus colonies which could be isolated in pure cultures as long as the physical signs were present, and then disappeared. Here was another new conception for me, a local consolidation of a base, afebrile, with no toxic or constitutional symptoms lasting over a period of two months, caused by a typical pneumococcus.

The problem gradually evolved until in the past four years the whole respiratory tract has been investigated, including acute rhinitis, the accessory sinuses, acute tonsillitis, tonsils after enucleation, laryngitis, whooping-cough, bronchitis, bronchiectasis, the various pneumonias, atypical consolidations, and chronic infections.

While the other secretions and excretions of the body, such as urine, gastric secretions, and exudates are carefully examined chemically, microscopically and bacteriologically, the sputum has been practically neglected, with the exception of the search for tubercle bacilli. It is true that the sputum has been repeatedly cultured in the investigations of rare and isolated cases; also in series of cases of *suspected influenza epidemics,* but I have been unable to find any systematic study of a series of cases of lung infections, by means of sputum cultures other than those made by Lord,[2] Wollstein,[3] Holt,[4] and Hastings and Niles.[5] Our knowledge of the respiratory infections is still drawn chiefly from necropsy findings, the results of which, owing to agonal and postmortem invasions of organisms from the mouth and the intestinal tract, have been justly questioned.

2. Lord, F. T.: Eleven Acute and Eighteen Chronic Cases of Influenza, Boston Med. and Surg. Jour., 1902, cxlvii, 662; Infections of the Respiratory Tract with Influenza Bacilli and Other Organisms, Their Clinical and Pathological Similarity, and Confusion with Tuberculosis, Boston Med. and Surg. Jour., 1905, clii, 537, 574.

3. Wollstein, M.: The Influenza Bacillus in Inflammations of the Respiratory Tract in Infants, Jour. Exper. Med., 1906, viii, 681.

4. Holt, L. E.: The Bacteriology of Acute Infections of the Respiratory Tract in Childhood, with Special Reference to Influenza, The Archives Int. Med., 1910, v, 449; The Bacteriology of Acute Respiratory Infections in Children as Determined by Cultures from the Bronchial Secretions, Tr. Assn. Am. Phys., 1910, xxv, 223.

5. Hastings, T. W., and Niles, W. L.: The Bacteriology of the Sputum in Common Nontuberculous Infections of the Upper and Lower Respiratory Tracts with Special Reference to Lobar and Bronchopneumonia, Jour. Exper. Med., 1911, xiii, 638.

A study of the results thus far obtained from necropsy material and sputum cultures will impress one at once with the great confusion and difference of opinion as to the etiological agent of the various respiratory infections. This is especially true of the infections of the upper part of the respiratory tract such as the nose, larynx, and bronchi.

The purpose of these papers is to demonstrate by means of sputum cultures an easy and reliable method of diagnosis of pulmonary infections, and to determine the etiological agent of these infections.

CAUSES OF INDIFFERENCE AND SKEPTICISM IN REGARD TO SPUTUM CULTURES

The usual conception of physicians in regard to sputum cultures is expressed about as follows: "You can't do anything with sputum cultures for you have such a mixture of organisms that you cannot tell whether one, all, or any of them bear any etiological relationship." Their idea is obtained from what they see on slides prepared and stained for tubercle bacilli, from sputum specimens indifferently collected and incubated at room temperature for hours, often days.

Then, too, the reports of the findings thus far have been so confusing that this opinion is rather strengthened. This is due to the character of mediums used. Anyone working with bacteriological problems of today knows that nearly all the work is done with special mediums, such as agar, broth or the various sugars enriched with fresh blood, blood serum, hydrocele fluid or the various exudates, all titrated accurately to a definite point for optimum growth, and that the ordinary laboratory medium is insufficient for the reason that organisms bearing an etiological relationship fail to grow or only so sparingly as to be overlooked or lost, and that only the ordinary contaminating organisms appear. It is only in recent years and by those working specially with influenza, that blood agar was used as a routine, and it is these workers who have obtained consistent results.

BACTERIA NORMALLY PRESENT IN THE RESPIRATORY TREE

Almost invariably, when one reports on the findings of a given respiratory infection, the question is asked as to the possibility of similar findings in normal cases.

Since the respiratory tree is an open channel, and since air may be laden with bacteria, one could readily assume that the lung is constantly inhabited by bacteria the same as the intestinal tract.

While we know that the air of high altitudes and at sea may be sterile, it has also been shown that the air of cities is laden with bacteria. One need only refer to the work of Hesse,[6] who found 350

6. Hesse, W.: Ueber quantitative Bestimmung der in der Luft enthaltenen Mikroorganismen, Arb. a. d. k. Gsndhtsamte, 1884, ii, 182.

organisms per ten liters of air in schoolrooms after dismissal; of Newman[7] and Miguel,[7] who found respectively 140 and 790 bacteria per ten liters in hospital wards; of Haldane,[7] who found 8,500 bacteria per ten liters in a ropemaker's workroom; of Thomson and Hewlett,[8] who found 164 organisms per ten liters in the thoroughfares of London; and of Sedgwick and Rockwell,[9] who found 200,000 organisms per ten liters in a dust storm 5 feet above the surface of a macadamized road.

The average amount of tidal air entering the lung being 500 c.c. and the average respirations per minute being about 18, the amount of air entering the respiratory tree is over 500 liters per hour. From these figures it can easily be deduced that one may inhale every hour 8,200 bacteria in the streets of London; 7,000 to 39,000 per hour in hospital wards; and 10,000,000 per hour in a dust storm on a highway.

It is therefore no surprise that most people have the conception of Rohrer,[10] who states that it is *selbstverständlich* that the nose and nasopharynx must show a large number of fungi, besides bacilli and cocci. This view has also been expressed more recently in an editorial[11] in the *British Medical Journal* under the caption "To Blow or Wash" in which the writer expresses the opinion that the civilized nose is one of the dirtiest organs of the body.

In a very modern text Ewart[12] assumes that the bronchi are inhabited by an "exceedingly varied flora" and are apt to contain any of the organisms present in the air, in the nasal cavities and accessory sinuses, the tonsils and the pharynx, together with the *Aspergillus Fumigatus, Oidium albicans* and other samples of lower vegetation, and also mentions the colon bacillus, the Friedländer bacillus, Löffler's bacillus, *Micrococcus tetragenus,* staphylococcus and others as among the "ordinary tenants" of the bronchi.

Because of these common beliefs, it seems desirable to bring in brief review the experimental facts.

BACTERIA PRESENT IN THE NORMAL NOSE

The nose and larynx have been cultured directly. Thomson and Hewlett,[8] Park and Wright,[13] Lewis and Turner[14] found that the

7. Hygiene and Public Health, Whitelegge and Newman, p. 7.
8. Thomson, St. Clair, and Hewlett, R. T.: Micro-Organisms in the Healthy Nose, Med.-Chir. Tr., Lond., 1895, lxxviii, 238.
9. Sedgwick, W.: Principles of Sanitary Science and the Public Health, 1902, p. 113.
10. Rohrer: Ref. in Centralbl. f. Bakt., 1888, iii.
11. Editorial: Brit. Med. Jour., 1895, i, 213.
12. Ewart, W.: System of Medicine, Allbutt & Rolleston, Ed. 2, 1909, v, 79.
13. Park, W. H., and Wright, J.: Nasal Bacteria in Health, New York Med. Jour., 1898, lxvii, 178.
14. Lewis, C. J., and Turner, A. L.: Suppuration in the Accessory Sinuses of the Nose: A Bacteriologic and Clinical Research, Edinburgh Med. Jour., 1905, xviii, 393.

healthy nose contains so few bacteria that if one rubs the septum and turbinates with a sterile platinum loop and inoculates this loopful of mucus on the surface of a blood agar plate 75 to 83 per cent. show no growth.

Ten cultures made by myself in this manner from hospital physicians and students showed 50 per cent. to be sterile, the other 50 per cent. showing only 1 to 5 colonies, all of which were nonpathogenic.

Surgical operations on the nose confirm the foregoing findings, since it is common knowledge that these wounds heal without sepsis.

Thomson and Hewlett made some experiments on the fate of organisms entering the nose. They placed a loopful of *Bacillus prodigiosus* on a spot of the turbinates. Cultures made from this spot up to two hours showed the following:

5 minutes, numerous.
15 minutes, much less.
60 minutes, diminished by 75 per cent.
80 minutes, frequently no bacteria.
2 hours, no bacteria could be found.

This experiment shows that the nose rapidly cleanses itself, probably in two ways: (1) by the outward movement of ciliated epithelium; (2) by the inhibitory or destructive action of the nasal secretion.

THE LARYNX

Cultures have also been made directly from the larynx. Jundell,[15] by means of a concealed swab on a sound, and with the aid of a laryngeal mirror, made direct cultures from forty-three cases, 90 per cent. of which were sterile.

TRACHEA AND BRONCHI

It is now generally agreed that the problem cannot be approached by using necropsy material, for it is found that the pneumococcus was found as often in normal lungs at necropsy as in the primary and secondary pneumonias.

Thus Dürck[16] found the pneumococcus present in the lung in 92 per cent. of thirteen necropsies on children free from any macroscopic or microscopic patches, while in forty-one cases of primary and secondary pneumonia he found the pneumococcus present in 84.6 per cent.

15. Jundell, I.: Ueber das Vorkommen von Mikroorganismen in den normalen Luftröhren, Skandin. Arch. f. Physiol., 1897-9, vii, 284.

16. Dürck, H.: Ueber die Aetiologie und Histologie der Pneumonie im Kindesalter, u. d. Pneumonie im Allgemeinen, Deutsch. Arch. f. klin. Med., 1897, lviii, 368.

Similarly, Norris and Pappenheimer[17] found the pneumococcus in 86 per cent. of twenty-eight cases affected with lobar pneumonia or other pulmonary lesions, while in fourteen cases of normal lungs, they found it in 93 per cent.

The reasons for this have been well brought out by the latter authors. They placed one half dram of a broth culture of *Bacillus prodigiosus* in the mouths of patients after death, and found that in 50 per cent. of cases this organism was present in the lung juice at necropsy.

One must also consider the agonal invasion of organisms from the bowel. Thus Ritchie[18] found the colon bacillus in 60 per cent. of his necropsies, in all of which he regarded the bacilli as secondary invaders. The proportion of cases in which they were present, and the number of colon bacilli found were strictly proportional to the number of hours that elapsed before the necropsy was made.

The same objection applies to slaughter-house material, since we cannot prevent the aspiration of fluid into the trachea and lungs during the death agony. Hence the problem has been approached by experiments with laboratory animals. In the laboratory experiments, the animals were killed by a knock, the trachea tied off immediately, the chest opened by cutting the diaphragm and lower ribs. Pieces of lung, mucous membrane, and mucus from the larynx, trachea, and bronchi were placed in bouillon and plated on agar. As a still further refinement the trachea ligature was placed before killing the animal, so that the fatal blow and the tying of the ligature would occur at the same time.

Hildebrandt,[19] Klipstein,[20] Göbell,[21] Barthel,[22] Becco,[23] and W. Müller[24] all came to the conclusion that the bronchi and lungs in normal animals are sterile.

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17. Norris, C., and Pappenheimer, A.: A Study of Pneumococcic and Allied Organisms in Human Mouths and Lungs after Death, Jour. Exper. Med., 1905, vii, 450.

18. Ritchie, W. T.: The Bacteriology of Bronchitis, Jour. Path. and Bacteriol., 1901, vii, 1.

19. Hildebrandt, G.: Experimentelle Untersuchungen über das Eindringen pathogenen Mikroorganismen von den Luftwegen und der Lungen aus, Beitr. z. path. Anat. u. Physiol., 1887, ii, 4111.

20. Klipstein, E.: Experimentelle Beiträge zur Frage der Beziehungen zwischen Bakterien und Erkrankungen der Athmungsorgane, Ztschr. f. klin. Med., 1898, xxxiv, 191.

21. Göbell: Ueber der Infection der Lungen von den Luftwegen aus, Dissertation, Marburg, 1897.

22. Barthel, T.: Ueber den Bakteriengehalt der Luftwege, Centralbl. f. Bakteriol. u. Parasitenk., 1 Abt. 1898, xxiv, 401, 433, 576.

23. Beco, L.: La flore bactérienne du poumon de l'homme et des animaux, Abstr., Centralbl. f. inn. Med., 1900, xxi, 846.

24. Müller, W.: Experimentelle und klinische Studien über Pneumonie, Deutsch. Arch. f. klin. Med., 1901, lxxi, 513.

In transferring these results to man, we have some direct evidence obtained by Kirchsteiner,[25] who made cultures on executed criminals.

To the foregoing we may add the theoretical considerations of F. Müller:[26]

1. Hemorrhagic infarcts of the lungs do not lead to abscess formation.

2. Surgically, lungs heal *per primam*.

3. In serous exudates into the pleura and pericardium, one often punctures the lung and nothing follows: if, however, one punctures a lung which contains bacteria, empyema may follow.

4. If bacteria were usually present in the lung, the same as in the mouth or intestine, it would be incomprehensible why mouth secretion or food which enters through insufficient closing of the glottis, is so dangerous and often leads to pneumonia (ex. bulbar paralysis).

All the experimental work is in harmony and from it one must conclude that the normal lung is sterile and that the bacteria are either filtered out in the nose or are destroyed after reaching the lung.

The nose with its deviated channel lined with ciliated epithelium, which rapidly moves the bacteria outward, is admirably adapted to protect the lung from dust and bacteria under ordinary conditions. If, however, the dust or bacteria content exceeds a certain stage, this protection is not sufficient, as is shown by the experiments of Hildebrandt.[19]

Clinically, we also have evidence that this protection is only relative, as is shown by the changes produced in the lung through the inhalation of dust in the various occupations, of which anthracosis is the most prominent example. Here we find that dust has penetrated the respiratory tree and is deposited in the lung, lymph channels, and lymph nodes. That the bacteria are destroyed after entering the lung is shown by the fact that normal lungs are sterile; also by the experimental introduction of bacteria, both virulent and avirulent, into the lung.

COLLECTION OF SPUTUM FOR SPUTUM CULTURES

We are all familiar with the cups sent to the clinical laboratory for the students to make stains for tubercle bacilli, and Gram stains. These cups contain the accumulation of the expectoration of from twelve to twenty-four hours, of post-nasal hawkings, mouth washes, and remnants of food. This mixture incubated at room temperature for about twelve hours is then labeled and sent to the laboratory for

25. Kirchsteiner: Personal communication to F. Müller.

26. Müller, F.: Der Keimgehalt der Luftwege bei gesunden Thieren, München. med. Wchnschr., 1897, xliv, 1382.

examination. These samples are valueless, and time-consuming even for an ordinary smear for tubercle bacilli, or for a Gram stain, since the most promising piece picked out will probably be a tenacious portion very likely derived from the postnasal space. It does not seem amiss to emphasize that for sputum cultures to give any results, the *sputum must be as carefully collected as blood or urine that is to be used for cultural purposes.* The sputum should be collected in a sterile dish, the mouth should be clean, and the patient interested in his case, and told that an attempt is being made to find the cause of his cough. The patient should be instructed to expectorate into the bottle or dish only what he is certain comes from his "boots," and also made to understand that very little is wanted, but that that little must be choice.

It is useless to give the dishes to the night nurse on the ward, for we all know how busy she is with the ordinary routine work, and how an attempt will be made to collect the sputum at a moment's notice like a specimen of urine.

The specimen when collected should be plated at once. If that is not possible, it should be kept on ice.

CHARACTER OF SPUTUM

It is impossible to predict the nature of the infection from the character of the sputum. If, however, one wants to get a good idea as to the nature of the sputum, the presence of casts, etc., there is no better way than to suspend it in a solution of salt or bouillon, in a Petri dish, and to wash it by means of a sterile platinum needle. The general impression is that the sputum from pneumonia is very tenacious, yet it is the most difficult sputum to wash. This is what one should expect, for the sputum from a pneumonic process is serosanguineous, while that from the bronchial tree is mucoid and tenacious. It is important to bear this in mind when culturing pneumonic sputum.

METHOD OF CULTURING THE SPUTUM

The sputum must be washed as thoroughly as its tenacity will permit, from six to ten successive sterile Petri dishes of bouillon or salt solution. (To observe the effect of this washing one need but make a smear before and after washing the specimen.) A portion was then macerated in a tube of bouillon and several loops transferred to fresh blood agar plates, then spread evenly over the surface by means of a bent glass rod which had been previously sterilized. To assist in the even distribution of the washed sputum on the plates, a small amount of sterile bouillon was added. These plates were incubated from twenty-four to seventy-two hours, and examined every day. All the colonies appearing in considerable numbers were subcultured and placed on all laboratory mediums, including blood agar, and the various sugars when these were necessary for differential diagnosis. In case of mixed infections the relative number of colonies were noted by actual and approximate counts. When an indefinite or negative result is obtained, it is important to make one or more subsequent

cultures. Even with the best of care one may fail to get the right kind of specimen. This is especially true in dealing with patients who are far below the average in intelligence. Then, too, certain stages of the disease yield better sputum specimens than others.

It is also important to use freshly prepared blood agar plates, as emphasized by Schottmüller.[27] The sugar medium was enriched with hydrocele fluid according to method of Holman,[28] as recently reported in the *Journal of Infectious Diseases*.[29] Atypical strains of pneumococci and streptococci were kept in stock for further study. All the pneumococcus and streptococcus strains were tested for their fermentative action on the various sugars, also their hemolytic power on fresh blood agar, their solubility by means of bile, and their ability to coagulate milk.

Many of the chronic cases were repeatedly cultured with a view of determining the constancy of the infection. It has been the belief of some that the flora is an ever-changing one. Some of these cases have been followed for four years and the type of the organisms and peculiarities in regard to hemolysis of blood, fermentation of sugars and coagulation of milk, have always been the same in a given case.

RESULTS OBTAINED

If the foregoing conditions in regard to collection of sputum and method of culturing are observed, it will be found that in acute conditions remarkable plates will result and that, as a rule, one organism will be present practically in a pure culture. This is especially true if the acute process occurs in a previously normal lung. If the lung is the seat of an old chronic process, an acute involvement will also show the type of infection in a practically pure state, but the infection may be mixed, owing to the presence of organisms causing the chronic process.

Table 1 gives a summary of the results of over 600 sputum cultures in 459 cases of infections of the respiratory tree. The figures in the table represent the number of cases of the particular infection as indicated on the left of the table, and the organism causing this infection as indicated at the head of the table; for example: bronchopneumonia; there were 42 cases and 56 cultures; 13 of the cases were caused by the pneumococcus, 1 case by the *Streptococcus mucosus capsulatus*, 1 by the pneumococcus and influenza mixed, 21 cases by the influenza alone, 1 by an atypical Friedländer, 1 by the *Micrococcus catarrhalis*. In 4 cases no result was obtained.

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27. Schottmuller, H.: Die Artuntersuchung der für den Menschen pathogenen Streptokokken durch Blutagar, München. med. Wchnschr., 1903, l, 849, 909.

28. Holman, W.: Personal communication.

29. Holman, W.: A Method for Making Carbohydrate Serum Broth of Constant Composition for Use in the Study of Streptococci, Jour. Infect. Dis., 1914, xv, 209, 227.

TABLE 1.—Summary of Results of Over 600 Sputum Cultures in 459 Cases of Infections of the Respiratory Tree

	Pneumococcus	Strept. Mucosus Capsul.	Atypical Pneumococcus	Pneumococcus and Influenza	Pneumococcus and Strep. tococ.	Streptococcus	Influenza	Influenza and Staphylococcus	Influenza and Pneumococcus	Bacillus Friedländer	Micrococ. Catarrhalis	Staph. Pyog. Aureus	Staph. Pyog. Albus	Staph. Cereus Albus	Bacillus Diphtheria	Bacillus of Hoffman	Bacillus Typhoid	Bacillus Coli	Bacillus Pyocyaneus	Bacillus of Bordet	Undiagnosed	No Result	No. of Cultures	No. of Cases
Bronchi and Lungs:																								
Lobar pneumonia	70	6	...	3	...	...	2	...	...	...	...	...	...	...	...	...	...	...	...	...	...	2	168	83
Bronchopneumonia	13	1	...	1	...	...	21	...	...	1	1	...	...	...	. .	...	...	...	...	...	. .	4	56	42
Acute bronchitis	17	2	3	1	...	2	20	1	...	...	1	...	...	...	...	...	...	...	...	...	...	...	49	47
Chronic bronchitis	3	...	...	1	...	...	5	...	...	1	...	...	...	...	1	...	...	...	...	...	1	1	17	13
Bronchiectasis	...	...	...	...	...	2	2	...	...	...	...	...	...	..	...	...	...	...	..	. .	...	...	8	4
Lung abscess	...	. .	..	...	...	3	...	...	...	...	...	...	...	...	. .	...	..	...	...	...	...	...	6	3
Asthma	...	...	...	...	...	...	1	...	...	...	...	1	1	2	...	. .	...	...	...	...	...	...	5	5
Mediastinal abscess	1	...	...	...	. .	. .	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1	1
Chronic infections	10	...	...	1	...	..	8	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	43	19
Tuberculosis	2	. .	1	...	2	...	2	...	1	...	...	...	...	3	...	...	...	...	...	...	...	10	21	21
Heart failure	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	3	4	4
Psychoneurosis	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	2	2	2
Larynx:																								
Laryngitis	17	...	3	...	...	...	7	...	...	...	5	..	1	3	...	...	...	...	...	...	...	2	39	38
Whooping cough	...	...	...	. .	...	...	. .	...	...	...	. .	...	. .	...	...	...	...	...	...	10	...	5	21	15
Nose, sinuses and tonsils:																								
Acute coryza	9	...	1	. .	...	2	6	...	...	...	3	1	2	2	...	3	. .	...	...	...	1	8	39	38
Sinuses	7	...	...	. .	...	2	4	...	...	...	...	1	...	...	...	...	1	...	...	...	...	7	29	29
Mastoiditis	1	...	...	...	...	8	...	...	...	...	...	...	...	. .	...	...	...	...	1	...	. .	1	11	11
Tonsils after dissection	4	...	1	...	...	35	7	...	...	...	...	7	2	...	...	...	...	...	...	...	...	4	60	60
Acute tonsillitis	1	...	...	...	...	7	...	...	...	...	...	1	...	...	...	...	...	...	...	...	...	...	9	9
Peritonsillar abscess	...	...	...	...	...	3	...	...	...	...	...	...	...	...	...	...	...	...	...	..	...	...	3	3
Otitis media	...	...	...	...	...	...	...	...	...	...	...	...	?	...	...	...	...	..	...	...	...	...	2	2
Normal nose	...	...	. .	...	...	...	...	...	...	. .	...	...	...	...	...	...	...	...	...	...	...	10	10	10
Total	...	...	...	. .	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	603	459

It will be seen from Table 1 that 95 per cent. of the infections are represented as single type infections either pure or in such preponderance that no doubt could be left as to the etiological agent. This large percentage is due to the fact that 87 per cent. of the cases were acute cases, and that in the 13 per cent. of chronic cases, many were also acute infections on top of an old process.

The material was drawn chiefly from the wards of the Johns Hopkins Hospital, many from my own private practice and that of friends. The hospital cases have the advantage of carefully worked out records, Roentgen ray examinations, and in many of the fatal cases, necropsy findings.

PNEUMOCOCCUS

It will be seen in Table 2 that while the pneumococcus is practically the sole cause of acute lobar pneumonia, it is also the etiological agent in 62.4 per cent. of all the respiratory infections below the larynx, and that together with the influenza bacillus it is the cause of 90.94 per cent. of all nontuberculous infections below the larynx.

It will also be seen that the pneumococcus is present in 55.5 per cent. of all cases of acute laryngitis and that with the influenza bacillus it is the cause of practically 75 per cent. of all acute infections of the larynx. In the infection of the head, nose, sinuses, tonsils and ear, the pneumococcus and influenza bacillus diminish in frequency, being present in 31.29 per cent. of cases, while the streptococcus assumes the predominant rôle, being present in 48.85 per cent. of the cases.

STREPTOCOCCUS

It will be seen that the streptococcus was present in only seven cases out of 244 cases cultured, or in 3.1 per cent. of all infections below the larynx; and that when present, it was associated with bronchiectasis, lung abscess, and acute bronchitis. This is somewhat at variance with the reports usually current in the textbooks. McPhedran[30] states that the streptococcus is found almost alone in infantile bronchitis of measles, gastro-enteritis and syphilis. It might be argued that this difference of findings was due to the fact that my material was limited almost entirely to infections in adults, and that in children the streptococcus plays a more important rôle. This, however, is not borne out by the findings of Holt,[4] and of Wollstein.[3] In sputum cultures of 257 cases of respiratory infections, Holt found the streptococcus "*rarely* as the predominant type, usually only in small numbers, in one half of the patients and in one third of the cultures taken."

30. McPhedran, A.: Modern Medicine, 1907, iii, 640.

In 138 necropsies, the streptococcus was found alone only *once* and present only 43 times.

To show how infrequently the hemolytic streptococcus type is found in the lungs, the following case is of interest:

TABLE 2.—Summary of the Number of Times Each Organism Was Found in the Various Respiratory Infections Enumerated in Table 1

	BRONCHI AND LUNGS	Per cent.	
138	Pneumococcus	62.44	=90.94
63	Influenza	28.5	
7	Streptococcus	3.16	
2	Friedländer	0.9	
2	Micrococcus catarrhalis	0.9	
1	Staphylococcus pyogenes aureus	0.45	
6	Staphylococcus cereus albus	2.71	
1	Diphtheria	0.45	
1	Undiagnosed	0.45	
221		100.00	
	LARYNX		
20	Pneumococcus	55.55	=74.96
7	Influenza	19.41	
5	Micrococcus catarrhalis	13.9	
1	Staphylococcus pyogenes albus	2.8	
3	Staphylococcus cereus albus	8.33	
36		100.00	
	HEAD		
24	Pneumococcus	18.32	=31.29
17	Influenza	12.97	
64	Streptococcus	48.85	
18	Staphylococcus pyogenes albus and aureus	13.74	
3	Micrococcus catarrhalis	2.29	
1	Undiagnosed	.76	
2	Hoffman's bacillus	1.52	
1	Typhoid and colon	.76	
1	Bacillus pyocyaneus	.76	
131		100.00	

Case 1.—E. B., aged 32, was admitted to the surgical ward Feb. 17, 1913, with a swelling in the neck beneath the left jaw which was incised and drained the following day. The temperature rose to 105 on the 20th, from which time it gradually fell, reaching 100 on the 26th, when it again rose to 105. He complained of severe headache and at times was irrational. On March 1 he was transferred to the medical side. At that time the blood culture was negative. On the 5th he was in a stupor, but could be aroused. The heart and lungs were not involved. A lumbar puncture was done but the spinal fluid was found negative. On the 10th he developed a fluctuating swelling on the left wrist, which was evacuated on the 12th. This pus contained the *Streptococcus pyogenes*. On the 12th a blood culture showed the *Streptococcus pyogenes*. Two subsequent cultures also showed the *Streptococcus pyogenes*. On the 15th, another metastatic, suppurating, phlegmon developed subcutaneously on the left foot. March 21 a similar indurated fluctuating mass was opened over the lower sacrum and a cup of pus was evacuated. The temperature at that time was 105. On the 24th and 26th the blood cultures were negative. March 27 an impairment developed in the right lower back, and the patient developed an acute lobar

pneumonia from which he died on March 31. On the 28th a sputum culture was made and a pure culture of a typical, green, inulin-fermenting pneumococcus was isolated. Both cultures, the streptococcus from the blood and the pneumococcus from the pneumonia, were passed through all the sugars and subjected to all the differential tests so that no mistake was possible in identification. If one ever had a right to expect a streptococcus process in the lungs it was in this case with repeated positive blood cultures of the *Streptococcus pyogenes* associated with multiple metastatic subcutaneous abscesses due to the same organism. Yet the terminal pneumonia was a typical pneumococcus affair.

INFLUENZA BACILLUS

The influenza bacillus was found in 28.5 per cent. of cases of nontuberculous infection below the larynx; in 20 per cent. of the infections of the larynx and 13 per cent. of the infections of the head.

While it is undoubtedly true that since the pandemic of 1889 and 1890 many of the so-called influenza epidemics have been found to be due to the pneumococcus, as has been shown by Curschman[31] and Pollak,[32] yet it certainly is a mistake to say "that in the last ten years it has sunken to the position of a saprophyte, found in bronchiectasis and tuberculosis, and that it is only sporadically dangerous and then only as a mixed infection."[33]

It will be much more true to say that the rôle of the influenza bacillus has steadily risen, and that, next to the pneumococcus, it is one of the most important infectious agents found in pulmonary lesions, that it is not only present in pandemics and epidemics, but also the cause of approximately 30 per cent. of the pulmonary lesions in interepidemic periods. If anything definite can be said about sputum cultures at all, it may be stated emphatically that the results are as convincing in regard to the influenza bacillus as they are for the pneumococcus. The cultures are so pure, the bacilli in such tremendous numbers, often 50,000 colonies on one plate made from a loopful of sputum, that no doubt can be left as to its etiological relation. The clinical process in these cases can, as a rule, be predicted in that the cases usually are not fatal, the toxemia less, and the pulse relatively slow.

These findings are in agreement with those of Lord,[2] who, in his influenza studies, found the influenza bacillus in pure cultures in 30 per cent. of the cases of lung infections in the interepidemic periods.

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31. Curschmann, H.: Pneumokokken Influenza, München. med. Wchnschr., 1909, lvi, 377.

32. Pollak, R.: Bakteriologische Befunde bei eitrigen Bronchitiden, Wien. klin. Wchnschr., 1908, xxi, 973.

33. Leichtenstern, O.: Influenza, 1912, Ed. 2, Quotation from Kongress-Zentralbl. f. d. ges. inn. Med. u. ihrer Grenzgeb.

MICROCOCCUS CATARRHALIS

R. Pfeiffer[34] first described a biscuit-shaped coccus, easily decolorized by Gram's stain, growing on ordinary mediums, but dying out quickly. More recently, Gohn and H. Pfeiffer[35] gave a detailed description of the cultural characteristics, virulence, differential diagnosis and frequency of occurrence. They studied 132 cases, and found the coccus pure only once, in a case of a child who died from profuse bronchitis.

Hastings and Niles[5] found it as the most frequent organism in their sputum cultures during the years of 1903 to 1910 inclusive. It was found pure in:

1 case of acute laryngitis.
4 cases of grip.
1 case of acute bronchitis.
1 case of asthma.

Their conclusion is that while "it is usually considered as a common, secondary invader, it may and probably does assume pathogenic properties."

Niles and Meara[36] found the *Micrococcus catarrhalis* in a case of bronchopneumonia complicated with arthritis and peritonsillar abscess.

It will be seen in Table 1 that the *Micrococcus catarrhalis* was found pure in

1 case of bronchpneumonia.
1 case of acute bronchitis.
5 cases of acute laryngitis.
3 cases of acute rhinitis.

The case of bronchopneumonia is especially worthy of consideration.

CASE 2.—K. P., aged 21, a sailor, was admitted with the following history: Two days before entering the hospital he was awakened from his sleep with dizziness, headache, and pain in chest. The pain in the side was more severe on breathing. Soon after onset he developed a cough with bloody expectoration.

The examination showed impairment of the right lower lobe, and suppression of breath sounds, indicating beginning consolidation of the right lower lobe. The leukocytes were 15,000. On the following day the breath sounds over the right lower lobe were suppressed, the note had a rather high pitch, and fine crackling râles were heard. The leukocytes had fallen to 8,700. The temperature on admission was 106, with a sharp crisis twelve hours later, remaining normal thereafter. Sputum cultures were made on the day of admission and on the two subsequent days. Each time a pure culture of the *Micrococcus catarrhalis* was found.

34. Pfeiffer, R.: Die Mikroorganismen, Ed. 3, 1896; personal communication to Kolle and Wassermann.

35. Gohn, A., and Pfeiffer, H.: Der Mikrococcus Catarrhalis (R. Pfeiffer) als Krankheitserreger, Ztschr. f. klin. Med., 1902, xliv, 262.

36. Niles, W. L., and Meara, F. S.: Lobar Pneumonia of Micrococcus catarrhalis and Bacillus coli communis, Am. Jour. Med. Sc., 1911, cxlii, 803.

There can be no question that this was a case of *Micrococcus catarrhalis* infection. It might be argued that the pneumococcus might have been absent in the sputum. Thus Hastings and Niles[5] found it absent in 65 per cent. of their cases of pneumonia, and Hastings and Boehm in 37 per cent. of their cases. My own findings with 168 sputum cultures in 83 cases of pneumonia show that in a true pneumococcus pneumonia the pneumococcus is never absent unless the patients are moribund and too sick to expectorate. In two such cases, no result was obtained by sputum culture.

STAPHYLOCOCCUS GROUP

It is interesting to note with what rarity staphylococci were found in infections of the lungs and bronchi. The *S. aureus* was found only once, and that in a case of asthma, the *S. albus* once, mixed with the *Staphylococcus cereus albus,* also in asthma. In infections of the larynx, the *S. aureus* was never found, the *albus* once, and the *cereus albus* three times. Even in infections of the head, in which one surely has a right to expect the staphylococcus if anywhere, it was found only 18 times in 151 cases; only once in acute coryza, only once in 29 cases of sinus infections in which the culture was made directly to blood agar at operation, and not once in 11 cases of mastoid infections.

The same observations were made by Lord, who did not find the staphylococcus once in pure culture in 183 cases of pulmonary infections.

Hastings and Niles[5] found the *Staphylococcus aureus* in 15 per cent. of their cases, and in pure culture in 10 per cent. as follows:

3 times in acute laryngitis.
7 times in chronic bronchitis.
6 times in lobar pneumonia.
3 times in bronchopneumonia.

The *Staphylococcus albus* was found in 6 per cent. of the cases, pure in 2 per cent., twice in grip, and twice in acute bronchitis. Their findings may be explained by the failure to use blood agar except where smears made from the sputum indicated Gram-negative organisms. Consequently, they found the influenza bacillus only once in seven years, 1903 to 1910; while Wollstein in 1906 found this bacillus in 17 out of 61 cases of bronchopneumonia; and Holt, in the years of 1908 to 1910 found it in 110 out of 257 cases, or 42 per cent. One of the reasons for their failure to find the influenza bacillus in smears is that all our counter stains are diffuse stains, and it is very difficult to distinguish delicate Gram-negative organisms from the débris on the slide. Hence the only safe guide is to use blood agar in all cases. It is also certain that nearly equally discordant results will be obtained

with the pneumococcus and streptococcus unless blood medium is used. The failure of Hastings and Niles to find the pneumococcus in the sputum of 43 out of 66 cases of lobar pneumonia, is a striking confirmation of the above view.

Holt found the *Staphylococcus aureus* as a mixed infection in 233 out of 257 cases of sputum cultures of children ill with pneumonia, bronchitis, tracheitis, and laryngitis. In 136 necropsies on children, it was also found alone 26 times; 8 times in pneumonia, 6 times in bronchitis, 8 times in pulmonary tuberculosis, and 4 times in non-respiratory cases. These necropsy findings hark back to the same discussion of the value of necropsy findings, and again illustrate the contention of Norris and Pappenheimer,[19] who found the pneumococcus as often in normal lungs at necropsy as in lungs affected by a pneumonic process. Similar results were found by Dürck in children.

The method of obtaining the sputum on swabs in children explains the mixture of the infection in their sputum cultures. From the beginning I avoided sputum obtained from children, little faith being placed in the source of the sputum obtained by introducing a swab into the child's throat and making it cough or gag. This idea is confirmed by Holt's findings,[4] in which he states that pure cultures were practically never obtained, and his conclusions are not at all convincing when he says, "what a mixed infection the acute pneumonia of early life is, the table shows strikingly."

TYPHOID BACILLUS

All the earlier investigations on the presence of typhoid bacilli in the consolidations of the lungs complicating typhoid fever were made on necropsy material and hence are subject to the same objections that are brought against all necropsy material on account of the agonal and postmortem invasions of bacteria into the lung.

Since 1887 sputum cultures were made by M. W. Richardson,[37] Stühlern,[38] Dieudonne,[39] Edel,[40] Glaser,[41] Jehle,[42] Rau[43] and others.

37. Richardson, M. W.: Upon the Presence of Typhoid Bacilli in Urine and Sputum, Boston Med. and Surg. Jour., 1903, cxlviii, 152.

38. Stühlern, V.: Beitrag zur Bakteriologie der Lobaren Typhus-Pneumonien, Centralbl. f. Bakteriol., etc., 1 Abt. Orig., 1900, xxvii, 353.

39. Dieudonné, A.: Zur Bakteriologie der Typhus-Pneumonien, Centralbl. f. Bakteriol., etc., 1 Abt., 1901, xxx, 481.

40. Edel, P.: Typhus Bacillen im Sputum, Fortschr. d. Med., 1901, xix, 301.

41. Glaser, F.: Die Bedeutung der Typhusbacillen bei Erkrankungen des Respirationsapparatus in Gefolge des Ileotyphus, und sein Auftreten im Auswerf, Deutsch. med. Wchnschr., 1902, xxviii, 772, 793.

42. Jehle, L.: Ueber den Nachweis von Typhusbacillen im Sputum Typhuskranker, Wien. klin. Wchnschr., 1902, xv, 232.

43. Rau, R.: Ueber das Auftreten Typhusbacillen im Sputum, und über ein typischen Fall von Pneumotyphus, Ztschr. f. Heilk., 1904, xxv, 385.

Edel examined eleven cases of bronchitis and found no typhoid bacilli.

Jehle examined twenty-two cases of uncomplicated bronchitis and found the typhoid bacillus present only four times and only once in pure culture.

Glaser reported two cases of pneumonia complicating typhoid fever in which the pneumococcus was present each time. The typhoid bacillus was also present, but to show how *nebensächlich* the typhoid bacillus is, he cites the following case:

> Man, aged 45, was admitted with typhoid fever and developed pneumonia eight days later, dying on the fifteenth day of his pneumonia. The sputum showed only pneumococci and no rods; on the fourteenth day of his pneumonia a lung puncture was made and typhoid bacilli were obtained in pure cultures. The patient died the next day, and the cultures made at necropsy showed numerous pneumococci with only occasional typhoid bacilli.

Edel reports one case of pneumonia accompanying typhoid fever in which he found the typhoid bacillus. He, however, does not exclude the pneumococcus or the influenza bacillus.

Stühlern reports two cases in which he found the pneumococcus and staphylococcus besides the typhoid bacillus.

Dieudonne reports one case in which he found chiefly pneumococci with only a few rods.

Jehle reports a case of lobular pneumonia complicating typhoid which he examined six times and found the typhoid bacillus five times in pure cultures. The necropsy also showed a great many typhoid bacilli.

My own observations are limited to three cases in none of which was the typhoid bacillus present. For instance:

> Case 3.—H. B. was admitted to the hospital January 12 with typhoid fever. The blood culture was positive for typhoid. Three days after admission he developed an impairment over the right lower lobe, with suppression of the breath sounds and numerous cracking râles. This condition persisted for one month and then cleared up. Ten days after admission the sputum showed a pure culture of the influenza bacillus.

Except for the case of Jehle, there are no cases on record, as far as I know, in which the typhoid bacillus seems to have been the only organism present. He is the only one who attempted to use blood medium (rubbed the surface of his plate with sputum and sterile horse blood). As was shown above, the influenza bacillus is the cause of 30 per cent. of all lung infections. It will thus be seen that in this percentage of cases, the typhoid bacillus might have been found alone, and yet not have been the etiological agent.

That one would expect the typhoid bacillus in the bloody serum of pneumonia complicating typhoid fever needs no elaboration, since one knows that in typhoid fever a bacteremia exists.

One must then come to the conclusion of A. Frankel,[44] who as early as 1899 stated that while not denying the possibility that the typhoid bacillus may cause a pneumonic process, he felt that the typhoid bacillus had only a secondary significance.

It must however be expected, that in cases of lung infarct followed by lung abscess the typhoid bacillus may be the only organism present. Flexner and Harris,[45] Robinson,[46] and Glaser[47] each report such a case.

In recent years considerable work has been done on the presence of typhoid bacilli on the tonsils and in the pharynx of patients ill with typhoid fever with special reference to the epidemiological aspect.

Manicatide[48] found it in 70 per cent. of cases, and Purjesz and Peri[49] in 20 per cent. of the cases.

Gaehtgens[50] found it only twice in thirty-two cases, in both of which there were typical throat ulcers.

Schutz[51] examined twenty-three cases and made cultures by using bile, Drigalski, and Endo mediums and found all negative. His conclusions are that typhoid bacilli are found only if ulcerations are present.

COLON BACILLUS

The colon bacillus was never found during life in this series of over 600 sputum cultures. There are, however, scattered reports in the literature.

In 1895, Hitzig[52] reported a case of lung gangrene from the sputum of which he isolated an atypical colon bacillus.

In 1908 Schrotter and Weinberger[53] reported a case of lobular pneumonia in which the colon bacillus was obtained, often in pure

44. Frankel, A.: Zur Lehre von den Affectionen des Respirationsapparatus beim Ileo-Typhus, Deutsch. med. Wchnschr., 1899, xxv, 231, 252.

45. Flexner, S., and Harris, N.: Typhoid Infection without Intestinal Lesions, Bull. Johns Hopkins Hosp., 1896-97, vii, viii, 259.

46. Robinson, G. C.: Rôle of Typhoid Bacillus in the Pulmonary Complications of Typhoid Fever, Jour. Infect. Dis., 1905, ii, 498.

47. Glaser, F.: Die Bedeutung der Typhusbacillen bei Erkrankungen des Respirationsapparatus in Gefolge des Ileotyphus, und sein Auftreten im Auswerf, Deutsch. med. Wchnschr., 1902, xxviii, 772, 793.

48. Manicatide, M.: Sur la recherche du bacille typhique dans le pharynx des malades de la fièvre typhoïde, Centralbl. f. Bakteriol., 1908, xlvi, 221.

49. Purjesz, B., and Perl, O.: Ueber das Vorkommen der Typhusbazillen in der Mundhohle bei Typhuskranken, Wien. klin. Wchnschr., 1912, xxv, 1494.

50. Gaehtgens, W.: Ueber die bakteriologische Typhusdiagnose auf Grund von neueren, in der praktischen Typhusbekampfung gesammelten Erfahrung, Berl. klin. Wchnschr., 1912, xlix, 296.

51. Schutz, F.: Ueber das Vorkommen von Typhusbazillen auf den Tonsillen Typhuskranker, Deutsch. med. Wchnschr., 1913, xxxix, 451.

52. Hitzig, T.: Beiträge zur Aetiologie der putriden Bronchitis, Arch. f. path. Anat. u. Physiol. u. f. klin. Med., 1895, cxli, 28.

53. Schrotter, H., and Weinberger, M.: Zur Kenntnis der Kolibazillose der Respirationsorgane, Wien. klin. Wchnschr., 1908, xxi, 505.

cultures. Nests of influenza-like bacilli and streptococci were also present during the first week, and it is not at all clear that the colon bacillus was the etiological agent.

In 1911, Niles and Meara[36] report an atypical case of bronchopneumonia, also complicated with diphtheria and erysipelas, in which they found the colon bacillus. No mention is made of the medium used, and unless blood agar was used the findings are in grave doubt.

One must conclude that the evidence for the colon bacillus is even less convincing than that for the typhoid bacillus.

DIPHTHERIA BACILLUS

The diphtheria bacillus was found once in a case of chronic bronchitis associated with an unidentified Gram-negative, motile bacillus. These organisms were found repeatedly over a period of one year in an abundant, green, mucoid sputum. The diphtheria bacillus was avirulent for guinea-pigs.

FRIEDLÄNDER BACILLUS

We hear much about the Friedländer bacillus, possibly because of the early discussion as to its relation to the etiology of pneumonia.

It therefore must seem rather surprising to find that it occurred only twice in 600 sputum cultures, nor was it present in all the subcultures made from isolated colonies accompanying the dominant organism.

Of the two cases in which it did occur, one was a case of chronic bronchitis in a woman of 60, the other a case of bronchitis, bronchopneumonia, and bronchiectasis. The dilated bronchi ended everywhere with cavities varying in size from grapeseed to hazelnut. In this latter case a pure culture of an atypical Friedländer was obtained, a Gram-negative, non-motile, capsulated bacillus, not fermenting any of the sugars, not changing milk to acid or producing coagulation.

UNITY OF INFECTION OF THE RESPIRATORY TREE

It will be seen from this study that the same organisms produce the infections of the upper and lower respiratory tract. If one reviews the reports thus far made on acute coryza, one would be led to believe that an entirely different flora is the cause of the nose infections from that of the chest infections. Yet it is contrary to what one should expect, and what the results from this series of cases indicate.

The character of the sinus infections supports the results obtained from acute coryza infections, for it would seem inconceivable that the infections of the sinuses should be the result of a different organism from that of the infections of the nose, from which they so frequently result and with which they have a direct anatomical connection.

VALUE OF SPUTUM CULTURES

It seems almost needless to say that all our knowledge of prophylaxis and specific therapy starts with our knowledge of the etiology of a given disease. Much of the diagnosis, the course of the disease and its complications are also dependent on a knowledge of its etiology. While the specific therapy may not immediately be at hand, we are at least laying the foundation-stones for such a therapy in the future.

It is also necessary that we should know the various pathological processes a given organism will produce. As a result of such studies we have learned that the influenza bacillus may produce lesions and clinical symptoms indistinguishable from those of the tubercle bacillus as shown by Lord.[2] In a subsequent paper we shall find that the pneumococcus also may produce chronic lesions extending over many years. It used to be the conclusion that all chronic chest conditions must be tuberculous whether the tubercle bacillus was found or not, and that is still the attitude to a large extent. By means of sputum cultures we may place against this negative evidence, the positive evidence of a definite organism in the sputum, which organism may explain the cause of the chronic condition. By following these cases over long periods with subsequent necropsy records, we are able to make an important contribution to our knowledge of pulmonary conditions.

If we now come to the practical, everyday interest of sputum cultures to physicians in practice, we have here, in a great many cases, a means of an early, positive diagnosis. In private practice the family wants to know, and often becomes very restless and may disturb one's peace and happiness unless such a diagnosis is made.

As examples:

Case 4.—A young man, aged 20, in a well-to-do family contracts an acute infection with severe cough, with a temperature rising to 103, somewhat of the remittent type. The lung findings point to a consolidation of a lower lobe. Pneumonia is the diagnosis. Fourteen days have elapsed and the crisis has not occurred, the cough is still very persistent, the temperature still reaches 103 in the evening, more remittent in type, tuberculosis is feared. A consultant is called; the diagnosis is still vague, we are still guessing. Tubercle bacilli, though looked for, have not been found. As a last resort, a sputum culture is requested. A pure plate of influenza bacilli with about 50,000 colonies results, one that you like to show around. Everybody is happy, a diagnosis has been made, and also a very reasonable prognosis.

Case 5.—C. S., aged 34, was admitted to the hospital March 18, eight days after onset of his illness, complaining of cough and tightness in chest. Five days before admission he stopped work and was feverish. Patient did not look especially ill, and on physical examination showed impairment of the left base with distant breath sounds and slight bronchovesicular breathing. One could not definitely say that patient had pneumonia. The leukocytes were 18,000. The physical signs were never definite, and on March 25 a diagnosis was made

of probable pleurisy with effusion on the left side. On March 29, on exploratory puncture, 10 c.c. of straw-colored fluid were obtained, not blood stained. The cell count was 3,300, 69 per cent. of which were of the small mononuclear type. The culture from this fluid was negative. This clear fluid, with extremely low cell count and negative culture, together with the atypical physical signs and the absence of toxic symptoms, put the diagnosis of pneumonia still further in doubt and strongly suggested tuberculosis. The blood culture was negative. Twelve sputum examinations were made for tubercle bacilli with negative results. A sputum culture was made on the 21st and a *bronchial cast was found* which when plated gave a beautiful plate of pure pneumococcus colonies. Further cultures were made on March 22 and 24 with the same results. The subsequent course was uneventful.

Here an absolute diagnosis of a pneumococcus infection was made on March 21 while eight days later, the clinical diagnosis of the nature of the process was still in doubt, and might have remained in doubt, with an unhappy diagnosis and prognosis to the patient. It is a simple enough matter in a large hospital, and especially in a public ward, to await developments and to make a diagnosis after the patient is well and discharged; but one can easily foresee the possibilities of such a case in private practice, in a nervous family, and with people of means.

It is also important that we should think in definite causes and effects. Blood cultures in lobar pneumonia are positive in about 30 per cent. of cases. In other pulmonary conditions they are practically always negative, and while we can record the symptoms and physical sign, our ultimate etiological diagnosis is usually guess work. In four consecutive necropsies, Park[54] found the influenza bacillus in pure culture in a case believed from the symptoms to have been due to the pneumococcus, and in two of the three cases believed to have been due to it, it was entirely absent. Except for these examinations, the clinician would have been of the opinion that he had clearly diagnosed the cases, while in fact, he was wrong in three out of four.

The following example illustrates how difficult it is to arrive at a true conclusion in regard to etiology unless sputum cultures are made.

Case 6.—W. A., aged 34, was admitted to the hospital Dec. 4, 1912. His illness began four years previously, following an exposure. The patient began to take cold easily, especially in the winter. The present attack began four weeks previously, with cough, expectoration, and smothering sensation in the chest. He expectorated large amounts of yellowish sputum, not blood-stained. He had no night sweats. On physical examination he showed a slight cyanosis and dyspnea, with palpable and audible rhonchi, but with no impairment of the percussion note. On auscultation there were numerous, coarse, bubbling, moist râles, which exploded in showers at the height of inspiration. The impression was that of an acute bronchitis, perhaps influenza. December 6 it was noted that the percussion note had a tendency to box-like resonance, and crackling râles were heard throughout both lungs. The summary of this examination indicated signs of acute bronchitis, probably associated with bronchpneumonia.

54. Park, W., and Williams, A.: Pathogenic Micro-Organisms, 1910, Ed. 4, p. 357.

An old emphysema was assigned as the cause of the cyanosis. Tuberculosis was ruled out, and either an influenza or a pneumococcus infection was suggested. The features during the remaining stay were dyspnea, cyanosis, cough and abundant expectoration. Generalized miliary tuberculosis was also suggested. December 10 the patient became an ashen hue and was covered with profuse perspiration. The lips were intensely blue, pulse was rapid and palpated with difficulty at the wrist. He was given oxygen inhalations. December 11, a high degree of emphysema was suggested, with bronchitis and bronchopneumonic patches. On the 13th the question of miliary tuberculosis was again considered. A probable influenza or pneumococcus infection was also suggested. The leukocytes varied between 8,000 and 10,000. The Calmette reaction was negative. Tubercle bacilli were not found. The Roentgen ray diagnosis was a dilated heart and aorta. The patient was discharged on the 23d, considerably improved. January 8 he was readmitted with the same physical signs as on previous admission, with the addition of purpura of the lower limbs. The diagnosis then was either chronic bronchitis and pneumonia, or a diffuse tuberculosis process in the lungs and the peritoneum. The patient died Jan. 10, 1913, probably from acute cardiac dilatation.

The anatomical examination showed the lungs salmon red in color, with the bronchi definitely enlarged, everywhere ending in cavities, varing in size from grapeseed to hazelnut. These cavities were lined with a smooth thin membrane and filled with a mucoid purulent secretion. In addition there were scattered red, raised, consolidated patches in all of the lobes. The bronchi everywhere were dilated, intensely injected, containing a mucoid purulent secretion. The heart was dilated with hypertrophy of the right ventricle and auricle.

A sputum culture was made on December 7 and repeated several times. The sputum was grass green, mucopurulent. Gram-negative bacilli were seen in smears. The plates showed a pure culture of a Gram-negative, non-motile, capsulated bacillus, belonging to the Friedländer group. This organism was atypical in that none of the sugars were fermented nor did it produce acid or coagulation in milk.

Here is a case, going through two hospital admissions, and a complete necropsy, without an etiological diagnosis. All the most probable infectious agents were suggested. The influenza bacillus, the pneumococcus, and the tubercle bacillus; and in view of the etiological agents suggested, a pathological process was pictured, from bronchitis and bronchopneumonia, to miliary tuberculosis.

If, however, we do know the etiological agent, and if we have a reasonably exact knowledge of what various processes a given organism may produce, we are in a fair position to visualize the pathological process.

The sputum cultures have also played an important part in our interpretation of Roentgen-ray plates of the chest. In 1911 Dunham, Boardman, and Wolman[55] made an extensive study of the markings in the lung. As a result of this study nearly all the chronic infections were diagnosed as tuberculous, regardless of whether the tubercle bacillus was found or not, or whether the tuberculin reactions were

55. Dunham, Boardman and Wolman: The Stereoscopic X-Ray Examination of the Chest with Especial Reference to the Diagnosis of Pulmonary Tuberculosis, Bull. Johns Hopkins Hosp., 1911, xxii, 229.

positive or not. The sputum cultures, however, gave us evidence of chronic influenza infections, and chronic pneumococcus infections, and it was shown that the chronic processes in the chest, due to these organisms, were indistinguishable by means of the Roentgen ray from those produced by the tubercle bacillus. The case cited in the introduction well illustrates this fact.

SUMMARY

1. Sputum cultures have been neglected because of the confusing results obtained, and the mixture of organisms found.

2. These results were due to unsuitable mediums and bacteriological methods.

3. By means of fresh blood agar plates, practically pure cultures can be obtained in 95 per cent. of the acute nontuberculous infections of the respiratory tract of adults.

4. The pneumococcus is the cause of 62.44 per cent. of all the nontuberculous infections below the larynx, and the influenza bacillus in 28.5 per cent. These two organisms cause 90.94 per cent. of the infections of the bronchi and lungs; 74.96 per cent. of the infections of the larynx, and 31.29 per cent. of the infections of the nose, throat and sinuses.

5. The streptococcus, contrary to common belief and textbook reports, is only rarely the cause of infections in the lungs, and when found, is usually associated with complications such as lung abscess, bronchiectasis, carcinoma, etc. In infections of the head, however, it assumes the predominant rôle, especially in the tonsils.

6. The *Staphylococcus aureus* was never found to be the cause of the acute infections of the lungs or larynx and only once was it found in acute coryza and acute sinusitis.

7. The *Micrococcus catarrhalis* may cause rhinitis, laryngitis, acute bronchitis and acute bronchopneumonia. It was found in pure culture in three out of thirty-seven cases of acute rhinitis, five out of thirty-eight cases of acute laryngitis, and in one each of acute bronchitis and primary bronchopneumonia.

8. The colon and typhoid bacillus rarely if ever produce acute lesions in the lung. The typhoid bacillus, however, may produce lung abscess following an infarct.

9. There is a unity of infection of the respiratory tree. The same organisms which cause the infections of the bronchi and lungs, also cause the infections of the nose, sinuses and larynx.

10. Sputum cultures afford us an easy, quick, and reliable method of diagnosis of pulmonary infections. Since blood cultures are negative in 70 per cent. of the cases of pneumonia and negative in nearly all of the other infections of the respiratory tree, it is practically the

only means of making an etiological diagnosis in the nonfatal infections.

11. This etiological diagnosis is necessary for a rational advance in prophylaxis and specific therapy. It is also a great aid in making an early diagnosis, and helps us to give an intelligent prognosis. This is especially desirable in private practice.

12. By means of these cultures we have also obtained a wider knowledge of what lesions a given organism may produce. These cultures show that each organism may produce a variety of acute lesions. Especially valuable information is afforded in that they show that the influenza bacillus and pneumococcus may produce chronic lesions extending over many years. This has an especial bearing on differential diagnosis, in regard to whether or not a given chronic chest condition is tuberculous.

1025 Madison Avenue.

THE RELATION OF THE ADRENALS TO THE PANCREAS *

F. C. MANN, M.D., AND DELLA DRIPS, A.B.
MAYO CLINIC, ROCHESTER, MINN.

It was noted at autopsy in a study of animals dying after the removal of both adrenals that the pancreas always presents a characteristic appearance. In color the gland is deep pink and contrasts quite strikingly with the faint white color of the normal pancreas. Because of their marked injection, it is possible to see all the vessels by transmitted light; even those leading to the small lobules stand out prominently. Histologically the islands appear prominent and the capillaries are engorged with blood. This appearance is only slightly simulated by that of the fatigue gland at the end of a long period of digestion. Since these changes in the pancreas were so marked and constant, we deemed them worthy of a separate investigation. This seemed especially desirable as accurate data in regard to the polyglandular theory are very scant. We found only one reference describing a seemingly similar condition of the pancreas. Sweet and Allen[1] noted that at necropsy of dogs from which the hypophysis had been removed the pancreas presented a striking red coloration like that seen at the height of digestion. Microscopic study, however, did not reveal any very marked changes.

Numerous experiments have been reported the results of which tend to establish the existence of a specific functional relationship between the adrenals and the pancreas. Hypotheses concerning this relationship are based on two facts: (1) That epinephrin produces glycosuria, and (2) that it decreases the flow of pancreatic juice. Investigation has failed to demonstrate that the glycosuric action of epinephrin is due to its primary effect on the pancreas. Lusk and Riche[2] conclude that the glycosuric action of epinephrin is due to vasoconstriction and not to inhibition of pancreatic function. Crowe and Wislocki,[3] who recently investigated the subject, conclude that carbohydrate tolerance is not modified in an adrenalectomized animal. The

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1. Sweet, J. E., and Allen, A. R.: The Effect of the Removal of the Hypophysis in the Dog, Ann. Surg., 1913, lvii, 485, 3 pl.

2. Lusk, Graham, and Riche, J. A.: Animal Calorimetry: Paper VIII. The Alleged Influence of the Adrenals on Diabetic Metabolism, THE ARCHIVES INT. MED., 1914, xiii, 673.

3. Crowe, S. J., and Wislocki, G. B.: Experimental Observation on Adrenals with Reference to Functions of their Interrenal Portions, Bull. Johns Hopkins Hosp., 1914, xxv, No. 284.

most comprehensive work on the relationship of the adrenals to pancreatic secretion has been done by Pemberton and Sweet.[4] They demonstrate that the injection of epinephrin decreases or inhibits the flow of pancreatic juice and that the removal of the adrenals increases the pancreatic secretion. From these observations they conclude that the adrenals have an inhibitory function over the pancreas. Edmunds,[5] in a study of the effects of epinephrin on the flow of pancreatic juice, concludes that the inhibitory action is due to the production of marked vasoconstriction.

Two general methods were used to study the effect of adrenal insufficiency on the pancreas:

A.—We performed a series of experiments in which the flow of pancreatic juice was carefully observed after various procedures on the adrenals. The experiments under this method may be considered in two general groups, the first comprising those experiments in which the flow was recorded in anesthetized animals, and the second those in which the flow was observed in unanesthetized animals.

B.—By means of special stains, we studied the histologic changes in the pancreas after these procedures.

METHOD A. FLOW OF PANCREATIC JUICE AND GROSS ANATOMIC CHANGES AFTER ADRENALECTOMY

Group 1.—The general procedure in Group 1 was as follows: All the animals were fasted for thirty-six hours prior to the time of experiment. They were carefully anesthetized with ether. Carotid blood pressure was usually recorded by a Huertle manometer; however, the blood pressure could be measured directly by means of a two way stop cock connected with a mercury manometer. All readings for blood pressure were taken from the mercury manometer. The flow of pancreatic juice was obtained by placing in the major pancreatic duct a cannula connected with a glass tube graduated in 0.01 cm. Readings expressed in cubic centimeters, were taken for fifteen-minute intervals. Blood pressure and flow of pancreatic juice were recorded hourly on an extension kymograph for a period of fifteen minutes. Normal temperature conditions were approximated as closely as pos-

4. Pemberton, Ralph, and Sweet, J. E.: The Inhibition of Pancreatic Activity by Extracts of Suprarenal and Pituitary Bodies, The Archives Int. Med., 1908, i, 628-647; Further Studies on the Influence of the Ductless Glands on the Pancreas, Ibid., 1910, v, 466; Sweet, J. E., and Pemberton, Ralph: The Induction of Pancreatic Activity by the Removal of the Adrenals, Ibid., 1910, vi, 536; Pemberton, Ralph, and Sweet, J. E.: Experimental Notes on the Influence of the Adrenals Over the Pancreas, Ibid., 1912, ix, 169.

5. Edmunds, C. W.: Further Study of the Relation of the Adrenals to Pancreatic Activity, Jour. Pharm. and Exper. Therap., 1910-11, ii, 559; The Antagonism of the Adrenal Glands Against the Pancreas, Ibid., 1909-10, i, 135.

sible by the judicious use of an electric pad. Since the passage of acid fluid from the stomach might prove to be a disturbing factor, the pylorus was usually ligated. In some experiments the minor duct was ligated to see if the free communication of the major and minor pancreatic ducts which is present in the dog was complicating results.

Series A.—In this series of five experiments, the flow of pancreatic juice was measured in normal anesthetized animals, the experiments usually extending over eight to sixteen hours. In general, our results corroborated those of Pemberton and Sweet. In one animal there was no flow; in the others there was a slight initial flow which increased as the blood pressure decreased. The following is a representative protocol:

Experiment 4 (*Series A*).—Oct. 5, 1914. Large male bulldog in good condition. Weight 36 pounds. Had fasted thirty-six hours. Etherized at 7:30 a. m. Apparatus arranged to record carotid blood pressure.

Time	Pulse	Resp.	Temp.	B. P.	Pan. Flow
7:45 a. m.	180	42	100.9	110	....
8:15 a. m.	180	42	98.2	110	....
8:15-8:30	...	..	.....	...	.04
8:30 a. m.	180	42	98.2	110	....
8:30-8:45	...	..	.....	...	.01
8:45-9:00	...	..	.....	...	.015
9:00 a. m.	156	48	97.6	105	....
9:00-9:15	...	..	.....	...	.005
9:15-9:30	...	..	.....	...	.000
9:30 a. m.	144	48	98.6	100	....
9:30-9:45	...	..	.....	...	.000
9:45-10:00	...	..	.....	...	.000
10:00 a. m.	150	42	97.3	90	....
10:00-10:15	...	..	.....	...	....
10:15-10:30	...	..	.....	...	.000
10:30 a. m.	130	46	97.4	85	....
10:30-10:45	...	..	.....	...	.000
10:45-11:00	...	..	.....	...	.000
11:00 a. m.	158	48	98.2	85	....
11:00-11:15	...	..	.....	...	.000
11:15-11:30	...	..	.....	...	.000
11:30 a.m.	132	36	98.8	90	....
11:30-11:45	...	..	.....	...	.05
11:45-12:00	...	..	.....	...	.04
12:00 m.	180	42	101.2	90	....
12:00-12:15	...	..	.....	...	.08
12:15-12:30	...	..	.....	...	.08
12:30 p.m.	180	42	101.4	90	....
12:30-12:45	...	..	.....	...	.000
12:45-1:00	...	..	.....	...	.000
1:00 p. m.	180	42	101.2	90	....
1:00-1:15	...	..	.....	...	.000
1:15-1:30	...	..	.....	...	.000
1:30 p. m.	180	48	100.6	105	....
1:30-1:45	...	..	.....	...	.000
1:45-2:00	...	..	.....	...	.005
2:00 p. m.	156	48	100.6	105	....
2:00-2:15	...	..	.....	...	.000
2:15-2:30	...	..	.....	...	.000
2:30 p. m.	156	48	101.6	100	....
2:30-2:45	...	..	.....	...	.000

Time	Pulse	Resp.	Temp.	B. P.	Pan. Flow
2:45-3:00	...	..	.....	...	.000
3:00 p. m.	180	60	101.8	100	....
3:00-3:15	...	..	.....	...	.000
3:15-3:30	...	..	.....	...	.000
3:30 p. m.	180	60	101.8	100	....
3:30-3:45	...	..	.....	...	.02
3:45-4:00	...	..	.....	...	.07
4:00 p. m.	180	60	101.1	80	....
4:00-4:15	...	..	.....	...	.06
4:15-4:30	...	..	.....	...	.08
4:30 p. m.	180	48	101.2	80	....
4:30-4:45	...	..	.....	...	.06
4:45-5:00	...	..	.....	...	.02
5:00 p. m.	180	48	101.9	76	....
5:00-5:15	...	..	.....	...	.05
5:15-5:30	...	..	.....	...	.03
5:30 p. m.	180	36	102.2	75	....
5:30-5:45	...	..	.....	...	.00
6:00 p. m.	180	36	102.4	60	....
6:00-6:15	...	..	.....	...	.06
6:15-6:30	...	..	.....	...	.05
6:30 p. m.	180	42	101.6	60	....
6:30-6:45	...	..	.....	...	.11
6:45-7:00	...	..	.....	...	.23
7:00 p. m.	180	42	101.0	50	....
7:00-7:15	...	..	.....	...	.08
7:15-7:30	...	..	.....	...	.14
7:30 p. m.	150	42	100.4	50	....
7:30-7:45	...	..	.....	...	.11
7:45-8:00	...	..	.....	...	.05
8:00 p. m.	180	36	100.1	45	....
8:00-8:15	...	..	.....	...	.29
8:15-8:30	...	..	.....	...	.07
8:30 p. m.	150	30	100.1	40	....
8:30-8:45	...	..	.....	...	.10
8:45-9:00	...	..	.....	...	.15
9:00 p.m.	180	36	100.6	38	....
9:00-9:15	...	..	.....	...	.12
9:20 p. m.	Animal died.				

Necropsy.—Performed immediately after death. Pyloric ligature had held, allowing no leakage. Thick fluid in stomach, which was markedly acid. No food material found above colon. There had been no hemorrhage. All organs appeared normal grossly. The weight of the pancreas was 45 gm.

Series B.—In this series of three experiments, after taking a control flow of pancreatic juice, the adrenals were quickly removed and the flow recorded until death. Our results again corroborate those of Pemberton and Sweet, although the increase we obtained is not so pronounced as they record. The following experiment is typical:

Experiment 2 (Series B).—Oct. 18, 1914. Large white bull in good condition. Etherized at 8:20 a. m. Considerable difficulty experienced in getting the cannula in the duct. Stomach not ligated. Normal pulse 210, respiration 42, temperature 99.2, and blood pressure 100 at 8:30 a. m.

Time	Pulse	Resp.	Temp.	B. P.	Pan. Flow
9:15 a. m.	168	42	99.2	85	....
9:30 a. m.	162	42	98.8	95	....
9:15-9:30	...	..	.....	...	.02
9:45 a. m.	Right adrenal was taken out.				

Time	Pulse	Resp.	Temp.	B. P.	Pan. Flow
9:30-9:45	...	..	.....	...	.04
9:51	Left adrenal was taken out.				
9:45-10:00	...	..	.....	...	.05
10:00 a. m.	162	42	98.4	95	....
10:30 a. m.	160	36	99.2	90	....
10:45-11:00	...	..	.....	...	.01
11:00 a. m.	142	32	99.4	100	....
11:00-11:15	...	..	.....	...	.04
11:15-11:30	...	..	.....	...	.03
11:30 a. m.	156	39	99.8	105	....
11:30-11:45	...	..	.....	...	.03
11:45-12:00	...	..	.....	...	.05
12:00 m.	180	36	99.6	105	....
12:00-12:15	...	..	.....	...	.09
12:15-12:30	...	..	.....	...	.11
12:30 p. m.	180	60	99.2	105	....
12:30-12:45	...	..	.....	...	.12
12:45-1:00	...	..	.....	...	.10
1:00 p. m.	180	60	99.2	65	....
1:00-1:15	180	36	99.2	60	.12
1:30-1:45	...	..	.....	...	.18
1:45-2:00	...	..	.....	...	.18
2:00 p. m.	180	40	99.8	50	....
2:00-2:15	...	..	.....	...	.26
2:15-2:30	...	..	.....	...	.22
2:30 p. m.	180	42	99.9	45	....
2:30-2:45	...	..	.....	...	.23
2:45-3:00	...	..	.....	...	.33
3:00 p. m.	160	36	99.8	40	....
3:00-3:15	...	..	.....	...	.30
3:15-3:30	...	..	.....	...	.25
3:30 p. m.	132	34	100.2	32	....
3:30-3:45	...	..	.....	...	.30
3:45-4:00	...	..	.....	...	.35
4:00 p. m.	162	42	100.4	35	....
4:05 p. m.	Injected small amount of adrenalin which increased the blood pressure, but decreased the pancreatic flow.				
4:16 p. m.	Injected nicotin which decreased blood pressure and pulse. Pancreatic flow decreased at first, but it soon quickened.				
4:22 p. m.	Animal died.				

Necropsy.—Performed immediately after death. No hemorrhage at the sites of operation. Pancreas grossly normal. The stomach contained about 150 c.c. yellow colored fluid, acid in reaction.

Series C.—In this series of twelve experiments one adrenal, usually the right, had been removed some days previously. The remaining gland was removed through a lumbar incision at the time of observation. Blood pressure was decreased but slightly by the adrenalectomy. In every experiment of this series the flow of pancreatic juice was increased after the removal of the remaining gland, but this observation is inconclusive, inasmuch as in many cases the increase was no greater than occurred in the control toward the end of the experiment. A typical experiment follows:

Experiment 7 (Series C).—Oct. 7, 1914. Large white bull bitch of gentle disposition whose weight was 11,942 gm. The right adrenal had been removed two weeks previously. The animal was in excellent condition. Fasted thirty-six hours prior to the experiment. Etherized at 8 a. m. and apparatus arranged to record blood pressure and pancreatic flow.

Table as follows:

Time	Pulse	Resp.	Temp.	B. P.	Pan. Flow
8:30 a. m.	180	60	98.2	120	....
8:45 a. m.	168	60	98.2	100	*
9:00 a. m.	168	66	98.2	95	....
9:00-9:15	...	..	.....	...	.04
9:15 a. m.	...	..	.....	...	.03
9:15-9:30	The remaining adrenal removed.				
9:30 a m.	174	60	98.0	95	....
9:30-9:45	...	..	.....	...	.03
9:45-10:00	...	..	.....	...	.03
10:00 a. m.	150	54	95.2	105	....
10:00-10:15	...	..	.....	...	.02
10:15-10:30	...	..	.....	...	.01
10:30-10:45	...	..	.....	...	.04
10:45-11:00	...	..	.....	...	.04
11:00 a. m.	150	54	95.2	115	....
11:00-11:15	...	..	.....	...	.04
11:15-11:30	...	..	.....	...	.04
11:30-11:45	...	..	.....	...	.035
11:45-12:00	...	..	.....	...	.055
12:00 m.	144	48	94.6	115	....
12:00-12:15	...	..	.....	...	.03
12:15-12:30	...	..	.....	...	.07
12:30-12:45	...	..	.....	...	.085
12:45-1:00	...	..	.....	...	.075
1:00 p. m.	150	48	96.0	110	....
1:00-1:15	...	..	.....	...	.08
1:15-1:30	...	..	.....	...	.07
1:30-1:45	...	..	.....	...	.085
1:45-2:00	...	..	.....	...	.075
2:00 p. m.	150	48	96.1	80	....
2:00-2:15	...	..	.....	...	.09
2:15-2:30	...	..	.....	...	.09
2:30-2:45	...	..	.....	...	.06
2:45-3:00	...	..	.....	...	.06
3:00 p. m.	120	24	96.1	55	....
3:00-3:15	...	..	.....	...	.05
3:15-3:30	...	..	.....	...	.06
3:30-3:45	...	..	.....	...	.06
3:45-4:00	...	..	.....	...	.08
4.00 p. m.	204	42	97.0	35	....
4:00-4:15	...	..	.....	...	.08
4:15-4:30	...	..	.....	...	.12
4:35 p. m.	Animal died.				

* Cannula inserted.

Necropsy.—Performed at once. Site of operation in excellent condition. No hemorrhage. No leakage through the pyloric ligature. Acid found in the stomach. All the other organs normal grossly. Weight of the pancreas 35 gm.

Series D.—In this series of five experiments, both adrenals were removed under sterile conditions, at the same time or at different operations. The pancreatic flow was studied after development of various degrees of adrenal insufficiency. We found that in an animal which had developed none of the symptoms of adrenal insufficiency

the pancreas, while active, showed no increase over the controls. In every animal studied after the development of the signs of insufficiency, the pancreas was found to be secreting but usually a scarcely appreciable amount. The following experiment is a record of the greatest flow obtained in this series:

Experiment 5 (Series D).—Adult male mongrel. On February 17, 1915, the left adrenal gland was removed. The animal quickly recovered from the operation.

Date	Time	Pulse	Resp.	Temp.	
3/1/15	5:30 p. m.	73	20	101.9	
3/2/15	8:00 a. m.	78	20	102.3	
	10:00 a. m.	Remaining adrenal gland removed.			
	3:30 p. m.	135	20	102.8	
	5:30 p. m.	142	21	103.2	
3/3/15	2:30 p. m.	142	20	103.2	
3/4/15	8:00 a. m.	150	22	103.1	
	9:30 a. m.	136	22	102.4	

Up to this time the animal appeared normal in every respect. Ate and drank regularly and showed no signs of muscular weakness.

3/4/15	2:55 p. m.	120	20	102.1	Normal data

ANIMAL ETHERIZED

3:00 p. m.	175	Cannula in carotid; blood pres., 80.		
4:15 p. m.	Cannula in main duct. Secretion slight, blood pressure, 50.			
Time	Pulse	Resp.	Temp.	Pan. Flow
4:15-4:30	...	..	.....	.24
4:30-4:45	...	..	.....	.17
4:45-5:00	...	..	.....	.15
5:00 p. m.	Animal bled to death from the femoral artery.			

Necropsy.—The pancreas was just beginning to become pink in color. The vessels were slightly congested.

Series E.—In this series of two experiments, after recording the normal flow of pancreatic juice, one adrenal was removed leaving the other intact. This procedure did not produce adrenal insufficiency, yet the increase in flow of pancreatic juice was just as great as in the instances in which both glands were removed. This fact is shown by the following experiment:

Experiment 11 (Series E). April 9, 1915. Adult male poodle dog. Weight, 8,200 grams. Was fasted for thirty-six hours prior to the operation. Pulse 130, respiration 24, temperature 101.2. Normal blood pressure 144. Etherized at 7:45 a. m.

Time	Pulse	Resp.	Temp.	B. P.	Pan. Flow
8:00 a. m.	142	36	101.0	144	....
	Abdomen opened and the minor pancreatic duct ligated. Cannula placed in the main pancreatic duct.				
8:30-8:45	...	..	.....	...	.02
8:45 a. m.	160	28	.....	116	....
	Left adrenal removed.			98	....
8:45-9:00 a. m.	...	..	.....	...	.025
9:00 a. m.	173	56	100.2	...	....
9:00-9:15	...	..	.....	...	.04
9:15-9:30	...	..	.....	...	.03
9:30 a. m.	216	52	100.0	...	....

Time	Pulse	Resp.	Temp.	B. P.	Pan. Flow
9:30-9:45	...	..	.....	...	.05
9:45-10:00	...	..	.....	...	.06
10:00 a. m.	184	48	100.1	...	....
10:00-10:15	...	..	.....	...	.06
10:15-10:30	...	..	.....	...	.10
10:30 a. m.	180	39	100.0	...	....
10:30-10:45	...	..	.....	...	.15
10:45-11:00	...	..	.....	...	.20
11:00	240	42	99.8	...	....
11:00-11-15	...	..	.....	...	.22
11:15-11:30	...	..	.....	...	.20
11:30 a. m.	240	54	104.0	85	....
11:30-11:45	...	..	.....	...	.22
11:45-12:00	...	..	.....	...	.23
12:00	240	48	104.6	85	....
12:00-12:15	...	..	.....	...	.23
12:15-12:30	...	..	.....	...	.30
12:30 p. m.	268	72	103.4	85	....
12:30-12:45	...	..	.....	...	.36
12:45-1:00	...	..	.....	...	.42
1:00-1:15	...	..	.....	...	.58
1:15-1:30	...	..	.....	...	.60
1:30 p. m.	270	52	103.8	50	....
1:30-1:45	Animal growing very weak.			40	....
1:45-2:00	...	..	.....	...	1.15
2:00 p. m.	240	38	104.2	...	....
2:00-2:15	...	..	.....	...	.89
2:16	Animal died.				

Necropsy.—Stomach almost empty, no free fluid, mucosa acid in reaction.

Group 2.—Method A. The flow of pancreatic juice in unanesthetized animals was studied in one series of experiments. In three other series the condition of the gland itself was studied.

Series F.—In this series of five experiments a temporary pancreatic fistula was established in an animal in which one adrenal had been removed. The fistula was made under sterile conditions by securely fixing a glass cannula in the major duct, after ligating the accessory duct. After observing the rate of pancreatic flow, and when the animal had recovered from the effects of the operation, the remaining adrenal was removed and the animal observed until death. These experiments were more or less unsatisfactory because of clotting of blood in the cannula, infection and necrosis of the duct at the point where the cannula was tied. In none of these experiments was there an increased flow of pancreatic juice after adrenalectomy; however, in none of the animals did the pancreas have the injected blood vessels and characteristic color as observed in the double adrenalectomized animal. The most important fact learned from these experiments was that the slight increase in flow noted in most of the anesthetized animals, after removal of the adrenals, was wholly insignificant when compared with the secretion of the gland during digestion. In the anesthetized animal the flow usually did not reach, and rarely exceeded, 0.05 c.c. in a quarter

of an hour, while in unanesthetized animals, of approximately the same size, during the digestive period the secretion was as much as 1 c.c. per minute. This fact is emphasized by the following experiment:

Experiment 4 (*Series F*). White and brindle male bulldog; old; very prone to fight. Right adrenal removed at 10 a. m., Oct. 31, 1914. Wound kept in good condition. Nov. 3, 1915, 10 a. m., the accessory pancreatic duct was ligated and glass cannula placed in main duct. Moderate secretion. Temperature of kennel maintained at 70 F. The animal drank milk at 12 m.

Date	Time	Pulse	Resp.	Temp.	Pan. Flow, c.cm.
11/3/15	2:15 p. m.	84	18	104.3	6.80
	5:00 p. m.	120	12	103.0	5.40
	6:10 p. m.	90	12	102.0	3.75
	6:15 p. m.	Animal drank milk.			
	8:30 p. m.	90	12	102.0	3.75
11/4/15	6:15 a. m.	140	12	103.0	1.15
	9:00 a. m.	...	..	.....	12.0
	9:30 a. m.	Remaining adrenal removed. There was no flow of pancreatic juice after this time.			
	11:00 a. m.	156	30	102.0	....
	12:00 m.	Animal drank milk.			
	2:16 p. m.	210	24	102.2	....
	5:30 p. m.	168	18	103.0	....
	9:15 p. m.	240	12	103.0	....
		Animal died during night.			

Necropsy.—The pancreas, aside from a slight congestion, seemed normal. Pancreatic cannula had ulcerated out of position.

Series G.—In practically all experiments, in anesthetized and unanesthetized animals, the contents of the stomach were found at autopsy to be acid. That the characteristic appearance of the pancreas, after removal of the adrenals, is not dependent on the acid condition of the stomach is proved by the results of this series of experiments. In five animals sodium bicarbonate, in large doses, was administered by a stomach tube every few hours from the time the last adrenal was removed until death occurred. While it is impossible to state that alkaline reaction of the stomach contents was maintained, it is certain that very little acid reached the duodenum. At necropsy in these animals the pancreas presented the same characteristic appearance as in other adrenalectomized animals.

Experiment 5 (*Series G*).—Dog B169. Feb. 12, 1915, right adrenal removed. Feb. 22, 1915, at 10:45 a. m. the left gland was removed. Sodium bicarbonate in 5 gm. doses was administered about every four hours until death on Feb. 28, 1915, at 8 a. m.

Necropsy.—Pancreas very dark, mulberry color; blood vessels markedly injected.

Series H.—In six experiments, after removing one adrenal the pancreatic ducts were doubly ligated and sectioned. At different times, varying from a few days to a few weeks, the remaining adrenal was removed. At autopsy of these animals the pancreas did not show the

characteristic color and injected blood vessels. In the experiments in which the last adrenal was removed shortly after ligation of the ducts the pancreas appeared normal; when a few weeks had elapsed between operations, the gland was converted into a hard, fibrous mass. The following experiment is an example:

Experiment 5 (Series H).—Dog B83. Small terrier about 8 months old. Nov. 10, 1914, at 11 a. m. both pancreatic ducts were ligated and right adrenal removed. At 4 p. m. seemed to have recovered from anesthetic and immediate effects of operation.

Nov. 11, 1915. Remaining adrenal was removed.

Nov. 12, 1915. Animal appeared normal.

Nov. 13, 1915. Developed the signs of adrenal insufficiency and died at 2 p. m.

Necropsy.—Site of the operations in good condition. Pancreas normal color, lobules stand out prominently; vessels not injected; firm; duct ligatures holding.

In one series of five experiments after the removal of both adrenals and the development of the moribund condition of adrenal insufficiency, the animals were bled to death. At necropsy the blood vessels of the pancreas were found to be still markedly injected with blood.

METHOD B. HISTOLOGIC CHANGES IN THE PANCREAS AFTER ADRENALECTOMY

Sections of the pancreas removed at necropsy from doubly adrenalectomized animals, when stained by the common methods, were rather characteristic. The islands were prominent, engorged with blood, and seemed more numerous than in sections from normal animals. The acinar tissue seemed to be decreased in amount. In order to study these changes to better advantage, the special differential stains of Bensley[6] were used. After the careful study of many sections from different animals, the following facts were ascertained:

1. There is a marked congestion which appears like a hypostatic congestion. Every capillary seems filled with blood.

2. There is very little or no zymogen present. When zymogen is present, it is found in the acinar cells which surround the islands or the small blood vessels. This loss of zymogen seems proportionate to the length of time that the animal has been moribund, the greatest loss being found in those cases in which the specimens were secured an hour or so after death. If the gland was removed immediately after death, there was less zymogen than if the animal was bled to death a few hours before it would otherwise have died. This loss of zymogen seems to be more closely related to the decrease in blood pressure than to the rate of pancreatic secretion. Evidently the storage

6. Bensley: Studies on the Pancreas of the Guinea-Pig, Am. Jour. Anat., 1911-12, xii, 297.

function of the acinar cells was affected more by the decreased food supply than by secretion.

3. The islands appear larger and more numerous than in sections from the normal animal. On close examination, after using Bensley's stains, some of these apparent islands were found to consist partially of island cells and partially of degenerated acinar cells. Some apparent islands were made up entirely of degenerated acinar cells.

4. The number and size of the islands, as well as the zymogen content, seem to depend on the length of time that the animal is moribund as well as on the length of time after death that the tissue is removed and fixed. Degenerative changes in the pancreas apparently occur slowly during the dying state and very quickly after death. The acinar cells show these changes before they appear in the island cells. The former lose their shape with the loss of zymogen, becoming more rectangular. The nucleus seems to move into the center of the cell. Both nucleus and cytoplasm stain much less intensely. Degeneration granules which take the acid stain appear in the protoplasm. All these changes make the acinar cells appear more like the island cells. As the changes proceed these groups of degenerated cells coalesce and a line of demarcation appears around them. The acini nearest the true islands seem most affected. They apparently join with the group of island cells to form a larger island. Isolated groups of these changed acinar cells increase the number of the apparent islands.

SUMMARY

The following facts may be taken to express a specific relationship between the adrenals and the pancreas:

1. The pancreas develops a characteristic pink color and its blood vessels are injected to a maximum degree after double adrenalectomy. This appearance is not dependent on the acid condition of the fluid in the stomach, but is probably associated with patent pancreatic ducts. The blood found in the injected pancreatic vessels cannot be removed by a free hemorrhage.

2. In an anesthetized animal the flow of pancreatic juice is always slightly increased after the removal of the adrenals.

3. There is a marked decrease in the zymogen content of the pancreas in adrenalectomized animals.

Against these data are the following:

1. The hyperemic condition of the pancreas does not take place until there is evidence that arterial tone is decreasing in the other organs.

2. The increased flow of pancreatic juice in the anesthetized animal after removal of the adrenals is but little greater than that which occurs in the normal anesthetized dog under the same conditions as regards blood pressure, etc., and it does not exceed the increase incident to the trauma from the removal of only one adrenal. Further, the flow is insignificant as compared to the flow during the process of digestion. In an unanesthetized animal, no increase in flow of pancreatic juice after removal of the adrenals is demonstrable.

3. The decrease in zymogen content is proportional to the time of low blood pressure.

4. All the changes found in the pancreas are proportionate to the length of time that the animal was in a moribund condition.

From a comparison of these data it is seen that all the changes found in the pancreas grossly, histologically and functionally, after removal of the adrenals, can be accounted for by the changes in general taking place in the organism as a whole, due to decreased blood pressure, decreased temperature, and the other changes incident to the development of the moribund condition. We have been unable to demonstrate any specific relationship between the adrenals and the pancreas.

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